Sensitivity of planted European beech saplings to soil drought

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Mit heißer Milch
Gummistiefel ausspülen,
diese hinter sich schütten,
so Spuren verwischen.

Günter Grass

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α	standardized regression slope
$\Psi_{L\,pd}$	pre-dawn leaf water potential
Ψ_S	soil water potential
ρ	Spearman's correlation coefficient
g_s	stomatal conductance for water vapor
A_{net}	net photosynthesis rate
ANOVA	analysis of variance
APE	atom percent excess
CEST	Central European summer time
CI	competition index
dpi	dots per inch
DSD	drought stress dose
FDR	frequency domain reflectometry
G	site-specific global radiation
HPLC	high performance liquid chromatography
ICP-OES	inductively coupled plasma optical emission spectrometry
IRGA	infrared gas analyzer
IRMS	isotopic ratio mass spectrometry
iWUE	intrinsic water-use-efficiency
LAR	leaf area ratio
LD	light dose
LMF	leaf mass fraction
m	amount
MRT	mean residence time
n	sample size
n.s.	not significant
nrBAI	relative normalized basal increment

NSC	non-structural carbohydrates
p	significance
P_{annual}	annual sum of precipitation
PACL	percent above canopy light
PAR	photosynthetically active radiation
PPFD	photosynthetically active photon flux density
r^2	adjusted coefficient of determination
$R_{below\,canopy}$	radiation reaching the forest floor
r/s ratio	root/shoot ratio
rBAI	relative basal area increment
REW	relative extractable water
RH_{air}	relative air humidity
RMF	root mass fraction
SD	standard deviation
SE	standard error
SLA	specific leaf area
SOC	soil organic carbon
SOM	soil organic matter
SRL	specific root length
SRTD	specific root tip density
STL	specific twig length
TDR	time domain reflectometry
VIF	variance inflation factor
VWC	volumetric water content

Summary

In view of climate change, the assessment of drought responses of European beech, particularly of planted saplings during the period of establishment in the stand, is of crucial importance for present silvicultural practice. In this thesis, experiments were performed to study the effects of soil drought on physiological, morphological and chemical traits of planted beech saplings. Further, a drought measure was developed allowing for quantitative evaluation of drought responses.

In a thinned Norway spruce stand, planted beech saplings were subjected to different levels of drought stress by throughfall manipulation during three growing seasons. Two further experiments were carried out with saplings growing in closed rhizotron boxes and in 20 l containers that also were exposed to different levels of soil water availability. Soil water potential was regularly measured in the rooted soil. For each sapling, the time course of soil water potential during the growing seasons was modeled based on FDR measurements. Cumulated soil water potential (*drought stress dose*, DSD) was employed as a quantitative measure for drought. The sensitivity of plant parameters as growth, physiology, morphology, nutritional status, non-structural carbohydrates, C allocation, etc. to soil drought was estimated using DSD as explanatory variable. Individual light exposure as well as allometric changes were considered as covariates.

DSD correlated with δ^{13}C in respiratory C fluxes and other plant parameters. As instantaneous drought responses, photosynthesis and stomatal conductance were reduced with decreasing soil water potential. A significant increase of mean residence times of recently fixed C in leaves and its delayed occurrence in soil respiration was observed with increasing drought, pointing to hampered within-plant assimilate transportation. Rhizosphere respiration was reduced by 50 % under most severe drought, while root/shoot-ratio increased. These observations explain the decrease of radial growth with increasing DSD and a shift in resource allocation under drought. The sensitivity of photosynthesis and growth to soil drought decreased during the three years after transplantation pointing to effective acclimation processes. Acclimation of root and shoot morphological parameters led to enhanced soil exploitation and reduced water loss by transpiration as indicated e.g. by increasing rooting depth and decreasing specific leaf area with increasing

drought. Non-linear drought responses were identified for root growth which was stimulated by moderate drought and hampered by severe drought. Changes in non-structural carbohydrates partly indicated C limitation under drought. Nutritional status of drought-stressed beech saplings mostly displayed hampered nutrient uptake. Rewetting led to a fast recovery of assimilate transportation as indicated by label-derived ^{13}C in respiratory C fluxes. Persistent effects of drought as revealed by differences in ^{13}C partitioning were ascribed to repair processes, but generally considered as small. Whereas some parameters were found to be indicative for recent (e.g. C/N-ratio in leaves) or former drought events (e.g. specific leaf area), others reflected the long-term average of soil drought (e.g. C/N-ratio in fine roots, abundance of mycorrhizal root tips).

It is concluded that beech saplings have a high resilience to soil drought due to efficient acclimation processes, especially of morphological traits. The drought stress dose as explanatory variable exhibited a considerable physiological relevance.

Zusammenfassung

Im Kontext des Klimawandels ist die Erforschung von möglichen Trockenheitsreaktionen der Rotbuche, insbesondere von gepflanzten Jungbäumen im Stadium der Etablierung im Waldbestand, von fundamentaler waldbaulicher Bedeutung. Im Rahmen der vorliegenden Arbeit wurden die Auswirkungen von Trockenheit auf morphologische, physiologische und chemische Parameter gepflanzter Jungbuchen untersucht. Weiterhin wurde ein quantitatives Trockenheitsmaß erarbeitet, welches eine allgemeingültige Darstellung von Dosis-Wirkungs-Beziehungen hinsichtlich Trockenstress ermöglichte.

In einem aufgelichteten Fichtenbestand wurden gepflanzte Jungbuchen durch Niederschlagsmanipulation über drei Vegetationsperioden verschiedenen Trockenheitsintensitäten ausgesetzt. Zusätzlich wurden zwei Experimente durchgeführt, im Rahmen welcher in Rhizoboxen bzw. Container gepflanzte Jungbuchen unterschiedlicher Bodenwasserverfügbarkeit unterlagen. Das Bodenwasserpotential im Wurzelraum wurde regelmäßig erfasst. Mittels kontinuierlicher FDR-Messungen konnte der zeitliche Verlauf des Bodenwasserpotentials für jede Jungbuche zudem individuell modelliert werden. Als Trockenheitsmaß wurde das kumulative Bodenwasserpotential (Trockenstressdosis, DSD) herangezogen und mit einer Vielzahl von Pflanzenparametern (Wachstum, Physiologie, Morphologie, Ernährungszustand, Gehalt nicht-struktureller Kohlenhydrate, Kohlenstoff-Allokation usw.) in Beziehung gesetzt. In die statistische Analyse wurden neben DSD gegebenenfalls die individuelle Lichtexposition sowie allometrische Aspekte als Kovariaten miteinbezogen.

DSD korrelierte mit $\delta^{13}C$ in Respirationsflüssen und anderen Pflanzenparametern; als sofortige Trockenheitsreaktion zeigte sich bei gestressten Pflanzen eine Verringerung von Photosyntheserate und stomatärer Leitfähigkeit. Ebenso wurde der Assimilattransport gehemmt, was durch eine Erhöhung der mittleren Verweilzeit rezenten Kohlenstoffs in Blättern und dessen zeitverzögertem Auftreten in der Bodenrespiration gezeigt wurde. Insgesamt wurde die Rhizosphärenrespiration unter starkem Trockenstress um 50 % reduziert, während das Wurzel/Spross-Verhältnis generell anwuchs. Diese Befunde erklären den gehemmten Radialzuwachs der Jungbuchen mit steigender DSD und spiegeln ein verändertes C-Allokationsmuster

unter Trockenstress wider. Die Sensitivität von Photosynthese und Zuwachs gegenüber DSD nahm jedoch im Verlauf der drei Jahre nach der Pflanzung stetig ab, was auf effiziente Akklimatisierungsprozesse, insbesondere hinsichtlich der Wurzel- und Sprossmorphologie, zurückzuführen ist: So ermöglichten z. B. die Erhöhung der Durchwurzelungstiefe und die Verminderung der spezifischen Blattfläche eine Intensivierung der Bodenerschließung bzw. eine Reduktion von Transpirationsverlusten. Nicht-lineare Reaktionen wurden beispielsweise beim Feinwurzelwachstum beobachtet, welches unter moderater Trockenheit stimuliert wurde, unter starkem Trockenstress jedoch zum Erliegen kam. Trockenheitsinduzierte Veränderungen im Gehalt nicht-struktureller Kohlenhydrate wiesen teilweise auf eine C-Limitierung der Pflanzen hin. Der Ernährungszustand der Jungbuchen spiegelte reduzierte Nährstoffmobilität unter Trockenbedingungen wider. Wiederbefeuchtung nach Trockenstress führte zu einer raschen Erholung der am Assimilattransport beteiligten Prozesse, was aus der Dynamik von Label-bürtigem ^{13}C in Respirationsflüssen gefolgert wurde. Persistente Trockenheitseffekte, die anhand von ^{13}C-Partitionierungsmustern aufgedeckt wurden, wurden Reparaturprozessen zugeschrieben, jedoch insgesamt als gering eingeschätzt. Die multivariate Analyse konnte einige Parameter als Indikatoren für rezenten Trockenstress (z. B. C/N-Verhältnis in Blättern) oder vorangegangene Trockenperioden (z. B. spezifische Blattfläche) identifizieren, während andere eher das Langzeitmittel der Bodentrockenheit widerspiegelten (z. B. C/N-Verhältnis in Feinwurzeln, Anteil mykorrhizierter Wurzelspitzen).

Insgesamt wiesen die Jungbuchen eine hohe Resilienz gegenüber Trockenheit auf, welche auf einem ausgeprägten Akklimatisierungspotential, vor allem der Morphologie, gründet. Zudem besitzt die Trockenstressdosis als erklärende Variable eine große physiologische Aussagekraft.

1 Synthesis

1.1 Introduction

1.1.1 Motivation

Drought is an abiotic stressor affecting plant performance and growth with consequences for forest ecosystems, silviculture and global carbon (C) cycle (e.g. Ciais et al., 2005; Granier et al., 2007; van der Molen et al., 2011; Sala et al., 2012). As predicted by climate models, the intensity and frequency of drought periods will likely increase during the next decades in many parts of the world, mostly because of altered temporal distribution of precipitation during the course of the year (Rowell and Jones, 2006; IPCC, 2007; Gerten et al., 2007; Ge et al., 2013). In Central Europe, the extreme drought during the summer months of 2003 has vividly demonstrated what forest ecosystems of the temperate zone will increasingly have to cope with: Productivity decline and increased mortality together with a significant derangement of the C fluxes in the system soil-plant-atmosphere emphasize the importance of this field of research (Ciais et al., 2005; Czajkowski et al., 2005; Leuzinger et al., 2005; Bréda et al., 2006; Rennenberg et al., 2006; Granier et al., 2007; Nikolova et al., 2008; Allen et al., 2010).

Forest conversion from conifer-dominated to mixed forests is a current practice (Kazda and Pichler, 1998; Bolte et al., 2009; Kölling et al., 2009), with European beech (*Fagus sylvatica* L.) playing a crucial role in Central Europe (Tarp et al., 2000). However, drought susceptibility of this species and its strategies to tolerate drought are still not fully understood. Reliable data on the behavior of young beech trees, especially planted saplings, are scarce. This is due to the lack of studies conducted under realistic silvicultural conditions that holistically consider drought responses of different plant compartments as well as the complexity of interactions between drought and other important site parameters, e.g. light conditions or competition. Therefore, assumptions on this species' suitability for the future climate are vague, as reflected by opposing views on drought susceptibility and future relevance of *Fagus sylvatica* (c. f. Rennenberg et al., 2004, and the rebuttal by Ammer et al., 2005).

Yet we lack reliable and referable measures for drought that allow a linkage between soil hydrology and plant drought responses. The soil-plant-relationship has only poorly been characterized so that it is difficult to compare and evaluate results of past studies on drought stress and thus to prognosticate risks of climate change for European beech based on existing water and climate models (Bréda et al., 2006; Schymanski et al., 2008).

1.1.2 Plant responses to drought

Water transport in the soil-tree-atmosphere continuum is driven by transpiration; i.e. a gradient of water potential (Ψ) between soil and different plant organs (van den Honert, 1948; Cruiziat et al., 2002; Aranda et al., 2005b). When soil water potential (Ψ_S) gets more negative while the soil is getting drier, this gradient decreases and, thus, water uptake at the soil/root interface is impeded. If water uptake does not counterbalance transpiration loss, we speak of *drought stress*.

Plants under drought stress aim to maintain water homeostasis by two general strategies: (1) restriction of water loss from the plant body and/or (2) promotion of water uptake (Kozlowski and Pallardy, 2002). These regulatory processes involve the adjustment of physiological traits as well as phenotypic plasticity (Nicotra et al., 2010) and are subsumed by the term *drought resistance* which itself comprises *drought avoidance* (plant maintains tissue water potential, e.g. by stomatal control or promotion of root growth) and *drought tolerance* (plant maintains its normal functions at lower tissue water potential, e.g. by osmotic adjustment) (Levitt, 1972; Kozlowski and Pallardy, 2002; McDowell et al., 2008; Jacobs et al., 2009; Lisar et al., 2012).
Drought responses are highly interdependent with other driving factors; they function on different time scales from seconds to years and concern different plant organs from cellular to whole-plant level. Whereas the behavior of the aboveground plant compartment has intensively been studied and characterized (Chaves et al., 2003; McDowell et al., 2008), our knowledge of belowground plant responses as well as whole-plant-interactions is poor, mainly due to operational difficulties together with a high natural variability (Vogt et al., 1996; Comas et al., 2000; Johnson et al., 2001; Majdi et al., 2005; Ostonen et al., 2005; Brunner and Godbold, 2007; Cudlin et al., 2007). Roots are the organs for water and nutrient uptake and therefore are the first plant organ that is confronted with soil water deficiency. Especially fine roots (diameter < 2 mm), which are relatively short-lived, serve as indicators for environmental stress (Cudlin et al., 2007); their plastic response upon drought is crucial for plant survival under adverse conditions. Although representing only a minor percentage of total plant biomass, fine roots further affect terrestrial C cycle by rapid turnover (McClaugherty et al., 1982; Nadelhoffer and Raich, 1992; Kätterer et al., 1995; Vogt et al., 1996; Ruess et al., 2003; Finér et al., 2011).

As plant water potential decreases, stomata closure, induced by turgor changes and/or biochemical signaling, results in a reduction of gas exchange between leaf and atmosphere (Hsiao, 1973; Christmann et al., 2007; Schachtman and Goodger, 2008). This down-regulation of transpiration rate is at the expense of photosynthesis since also CO_2 diffusion into the stomata is impeded; plants under

drought therefore face a trade-off between C gain and water loss which induces an adjustment of photosynthetic water use efficiency (Hsiao, 1973; Farquhar and Sharkey, 1982; Chaves et al., 2003). Proceeding stomatal closure reduces discrimination of $^{13}CO_2$ at the photosynthetic sites and thus increases $\delta^{13}C$ of formed photoassimilates (Dawson et al., 2002). This parameter has therefore been used as a valuable proxy for drought stress (e.g. Fotelli et al., 2003; Meier and Leuschner, 2008a).

Pattern and level of C allocation within the plant are altered by drought stress (van der Molen et al., 2011). Non-structural carbohydrates (NSC) which are important C reserves (Körner, 2003) can accumulate during drought due to nutrient deficiency but also get depleted in later stages of drought (McDowell, 2011). However, the significance of NSC for plant C balance is questionable and has been shown to vary widely also for different species, plant organs and age (Galvez et al., 2011; Ryan, 2011; Sala et al., 2012; Hartmann et al., 2013, and references therein) whereas some NSC may be indicative for osmoregulation processes (Kameli and Lösel, 1993). Another C sink is respiratory loss of CO_2 comprising maintenance and growth respiration (Eissenstat and Van Rees, 1994; Hanson et al., 2000; Janssens et al., 2002). Leaf and root respiration are affected by drought, *inter alia*, by changes of C availiability, growth rate and repair processes. Together with the respiration of root-associated fungi and other microorganisms that directly depend on C supply by roots, it is summarized as *rhizosphere respiration* (e.g. Kuzyakov, 2002) which responds sensitively to drought (Irvine et al., 2005; Borken et al., 2006). The tight temporal coupling of aboveground and belowground C fluxes has been shown to be disturbed by drought (Rühr et al., 2012), yet the complex interdependence of different aspects of C allocation is still not fully understood (Kayler et al., 2010; Brüggemann et al., 2011). However, a significant influence of drought is expected also on global C budget (Konôpka and Lukac, 2013; Sardans and Peñuelas, 2013; Kolb et al., 2013; Graf Pannatier et al., 2010; MacKay et al., 2012).

Drought can reduce plant growth since reduced turgor hampers cell division and expansion. Further, growth is restricted by nutrient deficiency and photosynthetic inhibition leading to C limitation (Hsiao, 1973; Ryan, 2011). However, one strategy to maintain water homeostasis is to improve water uptake. This can be achieved by increasing fine root production in order to enhance soil exploitation (Bauhus and Messier, 1999; Ho et al., 2005; Finér et al., 2011), especially the formation of deeper roots allows to reach moister soil layers (e.g. Bakker et al., 2009). A concomitant increase in root/shoot biomass ratio is an indicator of enhanced C allocation to the belowground compartment (Joslin et al., 2000; Bréda et al., 2006). However, limited carbohydrate and nutrient availability as well as reduced soil penetrability can restrict root production under severe drought (Joslin et al., 2000; Metcalfe et al., 2008; Bengough

et al., 2006). Drought increases fine root mortality (Janssens et al., 2002) but fine root productivity is variable. Under moderate drought, enhanced fine root production may counterbalance or even overcompensate root death, whereas severe drought can lead to the opposite (Sharp and Davies, 1979; Joslin et al., 2000; Leuschner et al., 2001; Gaul et al., 2008). Similarly, both increasing and decreasing fine root turnover, i.e. the ratio between stock and production rate, have been reported (Santantonio and Hermann, 1985; Mainiero and Kazda, 2006; Brunner and Godbold, 2007).

On a larger time scale, morphology of different plant organs can show a response to drought. For example, decreasing specific leaf area (SLA) with increasing drought exposition decreases transpiration loss in the long run (Hsiao, 1973; Hsiao and Acevedo, 1974; Kozlowski and Pallardy, 2002; Chaves et al., 2003). Other shoot architectural traits, e.g. twig ramification density, reflect drought avoidance strategies such as increased self-shading (Rodríguez-Calcerrada et al., 2008). Drought further promotes leaf senescence as it is associated with increased vulnerability to photo stress; premature leaf shedding is a possible strategy to reduce transpiration area under acute drought stress. Finally, xylem cavitation can irreversibly damage plant organs and eventually leads to death (Chaves et al., 2003; McDowell et al., 2008). The belowground plant compartment exhibits an even more prominent adaptability to drought: By acclimation of root morphology, plants optimize the cost/benefit-ratio (optimality theory, Bloom, 1985) of the root system (Eissenstat et al., 2000; Jackson et al., 2000; Leuschner et al., 2004; Ostonen et al., 2007; Bakker et al., 2009). For example, specific root length (SRL), i.e. fine root length per fine root biomass, is a proxy for this cost/benefit-ratio as it considers the benefit of resource acquisition (water uptake capacity) as well as the C-costs of construction and maintenance of living biomass (Eissenstat and Yanai, 1997; Ostonen et al., 2007). Hence, a positive correlation of SRL and drought was observed in many studies (e.g. Metcalfe et al., 2008; Montagnoli et al., 2012). Other important root morphological parameters are specific root tip density (SRTD) or root ramification density that are indicative for the efficiency of soil exploitation per amount of invested C (Hishi, 2007). Also mycorrhizal association enhancing the absorptive capacity of fine roots has been assumed to play a role for drought acclimation of plants (Davies et al., 1996; Shi et al., 2002).

Reduced soil water availability under drought coincides with a reduction of nutrient uptake which can be reflected in the plant's nutritional status (Peuke and Rennenberg, 2004, 2011; Maathuis, 2009). Generally, knowledge of drought effects on plant nutritional status is poor (Kreuzwieser and Gessler, 2010), since specific nutrient partitioning phenomena or osmoregulation processes alter nutrient composition which itself interacts with other plant parameters, e.g. P deficiency is assumed to control starch accumulation (Grossman and Takahashi, 2001).

Drought covariates

Drought is a stressor that interacts with other covariates among which light plays the most eminent role since photoinhibition is promoted by water deficiency (Chaves et al., 2003). Especially within forest stands that are typically patchy, light - and with that competition by neighboring plants (e.g. Guarnaschelli et al., 2012, and references therein) - is an important factor that determines the actual severity of a given drought level (Sack and Grubb, 2002; Sack et al., 2003; Quero et al., 2008; Rodríguez-Calcerrada et al., 2008; Gardiner et al., 2009; Robson et al., 2009). Further, light exposition influences the interdependency of shade and drought tolerance (e.g. Tognetti et al., 1994; Valladares and Pearcy, 2002; Huang et al., 2008; Puertolas et al., 2008; Valladares and Niinemets, 2008; Holmgren et al., 2012) and therefore has to be considered in silvicultural practice (Aussenac, 2000; Aranda et al., 2001). Also the belowground compartment is affected; e.g., light has been shown to influence rooting patterns (Ponge and Ferdy, 1997). In this context, competition also comprises belowground resource limitation concerning rooting space or nutrient uptake. Nutrient deficiency induced by drought and competition therefore are indistinguishably interlinked (Hsiao, 1973). Eventual synergistic effects of competition render this topic complex (Gouveia and Freitas, 2008; Mölder et al., 2010).

Acclimation denotes a long-term drought response, e.g. changes in fine root morphology towards enhanced soil exploitation (Kozlowski and Pallardy, 2002; Chaves et al., 2003) which occurs on a time scale of months to several years. Acclimation is explained by the optimal partitioning theory which implies that plants are able to respond plastically to environmental conditions, especially to limiting resources, by promoting those tissues or processes that are involved in the acquisition of the respective resource (Bloom, 1985). During establishment in the stand, concomitant allometric effects occur, i.e. effects that are triggered by ontogeny, e.g. changes in root/shoot-ratio, rooting depth or leaf morphology (Enquist and Niklas, 2002; Niinemets, 2010). Especially for plants in early stages of development, relative changes in allometry can be considerable as demonstrated e.g. for root/shoot ratio (Gedroc et al., 1996), so that they partly superimpose acclimation processes (Hertel et al., 2013, and references therein). For the interpretation of drought impacts, one has therefore to be aware that drought might alter the coherence of chronological age and ontogenetic stage.

1.1.3 Soil drought and drought stress quantification

Comparing different studies on drought is mostly difficult if not impossible. Drought stress is often only qualitatively presented in the literature by giving precipitation or irrigation amounts (e.g. Leuschner et al., 2004; Quero et al., 2008; Verdaguer et al., 2011). More relevant is a reference to volumetric water content (VWC) (e.g. Yang et al., 2007; Meier and Leuschner, 2008a; Rühr et al., 2009; Meier and Leuschner, 2010; Kuster et al., 2012).
However, the only parameter that provides adequate information on soil water availability is *soil water potential* (Ψ_S) in the rooted soil as it allows an energetic consideration of water movement in the system soil-plant-atmosphere (Vicca et al., 2012; Whalley et al., 2013). Measurement of Ψ_S - usually conducted with tensiometers and psychrometers - is not trivial and often time-consuming. Further, tensiometers are not suited for the drought range of interest ($\Psi_S < -0.3$ MPa), so that studies providing continuous data of Ψ_S mostly are in the range of mild drought (e.g. Gaul et al., 2008). A possible approach of this problem is modeling of Ψ_S - or diagnostic parameters such as relative extractable water (REW, e.g. Rühr et al., 2012) - based on continuous measurements of VWC. This can be achieved using pedotransfer functions; e.g. the van-Genuchten approach allows for the derivation of water retention curves based on substrate characteristics (van Genuchten, 1980). If small-scaled variations in soil water availability are to be assessed in heterogeneous substrates, e.g. natural forest soils with high stone content, this approach is disadvantageous as (1) the pedotransfer function reflects mean soil characteristics and (2) the measurement of VWC is critical itself because the signal of the most commonly used sensor types (frequency domain reflectometry (FDR) and time domain reflectometry (TDR)) is strongly influenced by organic matter content, air gaps, roots, clay content and bulk density (Muñoz Carpena, 2012).
Probably the best parameter reflecting plant water status is water potential in different plant organs (Myers, 1988; Vicca et al., 2012), especially pre-dawn leaf water potential ($\Psi_{L\,pd}$) or xylem potential. However, measurement of plant water potential is inappropriate for larger-scaled or long-term experiments as it is destructive and labour-intensive. Although measurements are occasional and therefore do not allow a continuous consideration, e.g. of evolving drought, $\Psi_{L\,pd}$ has been used as sole drought indicator (Tognetti et al., 1994, 1995; Peuke and Rennenberg, 2004; Jacobs et al., 2009; Robson et al., 2009). In such cases, it is difficult to quantify soil water availability, even if soil water content is additionally measured (e.g. Aranda et al., 2005a; Puertolas et al., 2008; Jimenez et al., 2009; Rose et al., 2009; Sanz-Pérez and Castro-Díez, 2010). The number of studies considering $\Psi_{L\,pd}$ together with Ψ_S is small (e.g. Veenendaal et al., 1995; Simpraga et al., 2011) leading to the fact that our understanding of the link between soil and plant water status is poor and thus under-represented in

soil-vegetation-atmosphere transfer models (Feddes et al., 2001; Bréda et al., 2006; Schymanski et al., 2008; Damour et al., 2010).
Short-term effects of drought stress, e.g. photosynthesis decline, are not necessarily of importance when considering effects on acclimation or growth on a larger time scale (Myers, 1988). Linder et al. (1987) used cumulated leaf water deficit, named *deficit time*, as a parameter reflecting drought exposure over a certain period. Fotelli et al. (2003) demonstrated that $\delta^{13}C$ in different plant tissues was correlated to time-integrated Ψ_S; in many cases, correlation yielded for 8-week-integrals was better than for shorter time-spans. Further, the time after which a drought period is reflected by morphological or chemical plant parameters differs among the plant organs (Löf and Welander, 2000; Ammer, 2003; Balandier et al., 2007; Sanz-Pérez and Castro-Díez, 2010; van der Molen et al., 2011).
Most studies on drought stress compare only one drought treatment with a control treatment (e.g. Tognetti et al., 1995; Gaul et al., 2008; Brunner et al., 2009; Rühr et al., 2009; Barthel et al., 2011; Peuke and Rennenberg, 2011; Schall et al., 2012). Such experiments ignore that drought responses of plants are not linear in many cases (Holmgren et al., 2012) and within a certain range, i.e. below a certain threshold of water availability, water is not limiting (Granier et al., 2007; Vicca et al., 2012). Thus, plant responses to drought can only be described adequately if a gradient of soil water availability is considered.
In summary, it can be stated that there is a need for a standardized, easy-to-determine yet significant measure for soil drought that allows a derivation of quantitative dose-response-functions for drought stress.

1.1.4 European beech under drought

European beech (*Fagus sylvatica* L.) dominates the potential natural vegetation in oceanic to subcontinental climates of Central Europe (Ellenberg, 1996). While silvicultural practice had promoted conifer species for centuries, climate change-motivated forest conversion has increased the importance of beech in our days (Knoke et al., 2008).
In comparison to other tree species, e.g. oak (Backes and Leuschner, 2000; Leuschner et al., 2001), European beech is considered as relatively drought-sensitive (Gessler et al., 2004; Ciais et al., 2005; Gessler et al., 2006; Zang et al., 2011a; Michelot et al., 2012) as also indicated by productivity decline of rear-edge populations under climate change (Jump et al., 2006; Scharnweber et al., 2011). Young beech trees have repeatedly been found to be more susceptible to drought stress than adult tress (Fotelli et al., 2001, 2002; Lendzion and Leuschner, 2008; Matjaž and Primož, 2010); particularly for planted saplings, the period of establishment in the stand is critical: Transplantation damages the

root system and additionally hampers its connectivity to the soil. This further increases the drought-vulnerability of such plants compared to that of natural rejuvenation (Burdett, 1990; Grossnickle, 2005; Kozlowski and Pallardy, 2002; Beniwal et al., 2011; Guarnaschelli et al., 2012).
Nevertheless, European beech has been reported to recover from drought stress and to survive severe drought events (Cermák et al., 1993; Tognetti et al., 1995; Gallé and Feller, 2007; Gallé et al., 2007). This ability has been attributed to its pronounced morphological plasticity (van Hees, 1997; Meier and Leuschner, 2008b; Konôpka, 2009) and efficient control mechanisms (e.g. Lemoine et al., 2002), but findings are often contradictory (e.g. Mainiero and Kazda, 2006).
Incompatibilities in experimental designs together with the lack of a uniform drought stress quantification hamper the synoptic evaluation of past studies and cause a vague perception of potential risks of increasing drought for European beech (Rennenberg et al., 2004; Ammer et al., 2005). Applicability of many studies to silvicultural practice is often poor as site-specific characteristics, especially light conditions and competition, influence the drought responses of this particularly shade-tolerant species in forest stands (Eschrich et al., 1989; Welander and Ottosson, 1998; Löf, 2000; Löf et al., 2005; Coll et al., 2004; Curt et al., 2005; Kunstler et al., 2005; Jarcuška, 2011; Jarcuška and Barna, 2011; Schall et al., 2012). One has to keep in mind that *F. sylvatica* has an extremely broad genetic variability with many ecotypes that potentially differ in drought tolerance (Peuke et al., 2002; Peuke and Rennenberg, 2004; Schraml and Rennenberg, 2002; Czajkowski and Bolte, 2006; Rose et al., 2009; Stojnic et al., 2012; Sánchez-Gómez et al., 2013). However, genetic variability does not always exceed morphological plasticity within populations (Meier and Leuschner, 2008b; Wortemann et al., 2011).

1.2 Aims and general strategy

This thesis aims at a characterization of the drought responses of planted European beech saplings by means of physiological, morphological and chemical traits. Further, drought responses are to be linked with a quantitative drought measure in order to derive reliable statements on the fitness of European beech under drier climatic conditions.

The general strategy comprises the following keynotes:

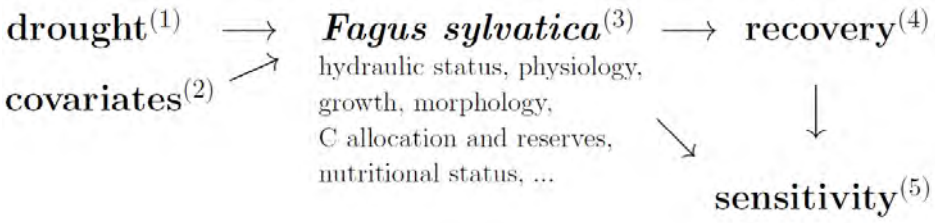

Figure 1.1: Keynotes of the general strategy. Further explications in the text.

(1) European beech saplings are to be subjected to different levels of soil drought as quantified by soil water potential and cumulated soil water potential which are assessed individually and serve as explaining variables on an interval scale (drought gradient).

(2) If necessary, light as important covariate is to be considered in statistical data analysis. Therefore, the individual light regime is quantified in analogy to soil drought. Further, allometric changes are to be considered in statistical models.

(3) To match a silviculturally relevant scenario, underplanted beech saplings from a typical reforestation site are considered. To do justice to the complexity and interdependence of possible drought responses, a multitude of plant parameters is to be assessed, from short-term to long-term responses of all plant compartments including physiology, morphology and chemical aspects.

(4) Besides the question of whether and how a plant responds to drought stress, the ability to survive and to recover from drought events is crucial for the continuance of its population under future climate scenarios and has therefore to be examined.

(5) Obtained dose-response functions provide a starting-point for the risk assessment of climate change for European beech saplings.

1.3 Materials and Methods

Overview

A **throughfall manipulation experiment (study I)** simulated large gradients in soil water availability in a typical reforestation situation with planted beech saplings. The influence of drought stress on a multitude of plant parameters was assessed during three growing seasons (see chapter 2). Drought responses were further characterized by assignment to 'response categories' (see chapter 3).

To assess the effects of drought stress on the dynamics of root growth and C fluxes within a growing season, a **rhizotron experiment (study II)** was conducted on the same site in 2010. This experiment was characterized by controlled conditions with saplings growing in closed boxes (see chapter 4).

A **labeling experiment (study III)** was carried out in 2011. Five-year-old beech saplings were subjected to defined levels of drought and subsequently rewetted. Pulse labeling was conducted before and after rewetting with the aim to trace the fate of recent assimilates and to evaluate the ability of beech saplings to recover from drought stress (see chapter 5).

An overview of assessed parameters and sample sizes in the different experiments is provided by Tab. 1.1.

1.3.1 Throughfall manipulation experiment (study I)

Study site

The throughfall manipulation experiment was performed on a thinned Norway spruce (*Picea abies* [L.] Karst.) stand (140 stems per hectare, tree age 145 years) in North-East Bavaria, Germany (50° 8' N, 11° 52' E, 770 m a. s. l.). The soil at the stand has evolved on deeply weathered granites of Ordovician age and is classified as a haplic Podsol with a sandy to loamy texture. The organic layer is well stratified into the sub-horizons Oi, Oe and Oa and corresponds to a moder humus form with a thickness ranging between 6 and 13 cm. Mean annual air temperature at the site is 5.3 °C and mean annual precipitation accounts for 1160 mm. The understorey vegetation mainly consists of *Calamagrostis villosa* [Chaix] J. F. Gmel., *Deschampsia flexuosa* L., *Vaccinium myrtillus* L., *Sambucus racemosa* L., *Rubus spec.* and *Senecio ovatus* Willd. For further site and soil characteristics see Gerstberger et al. (2004) and Hentschel et al. (2007).

Table 1.1: Overview of assessed plant parameters and sample sizes in the different experiments. Numbers represent the number of individual beech saplings considered, numbers in parentheses denote the total number of measurements. Asterisks indicate the data sets subjected to incremental multivariate regression analysis (see chapter 3).

parameter	**study I**			**study II**	**study III**	**see figure/table**
	2009	**2010**	**2011**	**(2010)**	**(2011)**	
pre-dawn leaf water potential			56(82)			Fig. 1.3
net photosynthesis rate		22(88)	46(323)	24(144)	30(60)	Fig. 2.4, 2.5, 4.3; Tab. 2.3, 5.2
stomatal conductance		22(88)	46(323)	24(72)		Fig. 2.5, 4.2; Tab. 2.3
δ^{13}C in leaf and soil respiration					30	Fig. 5.2; Tab. 5.2
δ^{13}C in buds			42*			Fig. 1.4, 3.1a; Tab. 3.2
δ^{13}C in leaves and fine roots			6			Fig. 1.4
rhizosphere respiration				24(288)		Fig. 4.4, 4.5
BAI	81	81	81			Fig. 1.5, 2.3; Tab. 2.3
r/s-ratio			30*	24	30	Fig. 2.2; Tab. 3.1, 4.1, 5.1
fine root growth				24(192)		Fig. 4.6
SLA		36	60*			Fig. 2.4; Tab. 2.3, 3.1
LMF, LAR, total tiller length, twig ramification density, bud weight			60*			Tab. 3.1
SRL/average fine root diameter, SRTD	27	36	59*	24		Tab. 2.3, 3.1, 4.1
rooting depth			30*			Fig. 2.6; Tab. 3.1
mycorrhizal root tips			60*			Fig. 3.1c; Tab. 3.1
fine root diameter class length				24		Fig. 4.7
label MRT in leaf respiration and occurrence in soil respiration before and after rewetting					30	Fig. 5.3 - 5.5; Tab. 5.2
^{13}C mass balance after recovery					30	Fig. 5.6, 5.7; Tab. 5.2

Plant material

All beech saplings (*Fagus sylvatica* L.) that were used in this study were of a colline to sub-montane provenance (origin code 81012). The plants were obtained from a local nursery and were bare-rooted. Three-year-old saplings have been planted on the study site in autumn 2008 in a density of 2500 plants per hectare.

Manipulation of soil water availability

The forest site was subdivided into nine plots with an area of 400 m^2 each. In three replications each, three regimes of soil water manipulation were applied: On irrigated plots, non-limited soil water availability was assured throughout the year by an automatic sprinkler system. Deionized water was sprayed on the plots whenever Ψ_S fell below -0.015 MPa. On throughfall exclusion plots, wooden roof constructions (height: 2.5 - 3 m) were closed with transparent panels for 8 weeks in 2009 (June 15^{th} through August 15^{th}) and for 12 weeks each in 2010 and 2011 (June 10^{th} through September 10^{th}, each). Three control plots without throughfall manipulation reflected natural conditions on the study site.

Concept of drought quantification

A FDR soil moisture sensor (10 HS, Decagon Devices, USA) was installed in 10 - 20 cm mineral soil depth in the rooting zone of each beech sapling. Sensor signals [mV] were hourly logged during the growing season from 2009 to 2011 (Fig. 1.2 a). For a soil water potential of -0.05 MPa, the signal of 50 installed FDR probes varied between 600 and 1060 mV (mean: 862 mV) which is explained by natural soil heterogeneity (distribution of macro pores and stones, roots, air gaps, etc.). Thus, it was not possible to employ the intrinsic calibration function provided by the manufacturer to calculate VWC directly from the sensor signal.
Individual sensor calibration was therefore performed *in-situ* by additionally measuring Ψ_S at the same soil depth. We used a portable tensiometer (T5 Tensiometer, UMS, Germany) for $\Psi_S >$ -0.3 MPa; $\Psi_S <$ -0.3 MPa was quantified by analysing a soil sample with a psychrometer (WP4, Decagon Devices, USA) in a climate chamber at 20 °C with up to 20 measurements per sensor and year. Individual FDR sensor calibration was performed via spline regression of measured Ψ_S and the corresponding sensor signals in mV (Fig. 1.2 b). The calibration splines were calculated using the *stats*-package in R 2.13.0 (command `smooth.spline()`, R Development Core Team, 2009): For m measurements, the degree of freedom of the splines was $\frac{m}{2}$. The time course of sensor signals was then directly converted into $\Psi_S(t)$ (Fig. 1.2 c).
As a measure for soil drought during the growing season, we calculated cumulated soil water potential in the period between 1^{st} of May and 1^{st} of October of each year by integration of $\Psi_S(t)$. In the following, this parameter is referred to as *drought stress dose* (DSD) given in the unit [MPa d] with

$$DSD = -\int \Psi_S\,(t)\; dt$$

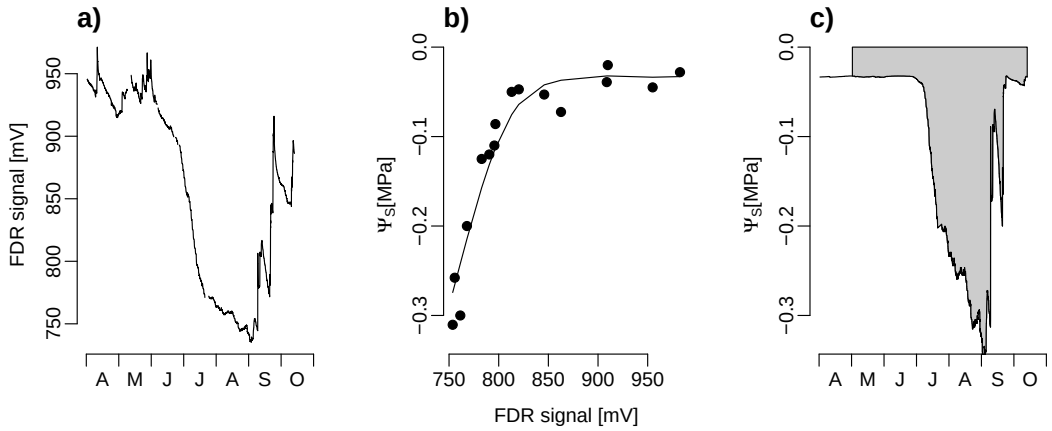

Figure 1.2: a) Time course of the FDR sensor signal of one selected beech sapling during one growing season. **b)** Correlation of measured Ψ_S with the corresponding sensor signal and the individual calibration spline. **c)** Time course of Ψ_S calculated based on sensor signal and calibration spline; the gray area represents the *drought stress dose*.

Light conditions and competition

As an important covariate influencing plant growth, the light regime was assessed for each plant by hemispherical photography at the top height of the saplings in 2011 (WinSCANOPY DSLR system, Regent Instruments Inc., Canada). The individual light dose (LD) during the growing season was then calculated based on the course of site-specific global radiation above the canopy (data from local climate measurement tower) during the considered growing seasons.
Additionally, competition by mature spruce was quantified by calculating a modified Hegyi-competition index (CI) (c.f. Hegyi, 1974) for every beech i with j competing spruce trees as follows:

$$CI_i = \sum_{j=1}^{n} \frac{DBH_j}{dist_{ij}}$$

where DBH is the diameter at breast-height of the competing tree [cm] and $dist$ is the distance between beech sapling and competing tree [m].

Leaf water potential

Pre-dawn leaf water potential ($\Psi_{L\,pd}$) was measured according to Scholander et al. (1965) in order to assess the saplings' individual water status and to check the plausibility of measured/modeled Ψ_S. Leaves of side-branches of randomly chosen saplings were clipped before sunrise (3.00h CEST) and immediately analyzed using a Scholander pressure chamber (Plant Water Status Console 3005

series, Soilmoisture Equipment Corp., USA). 82 measurements of $\Psi_{L\,pd}$ were carried out on 6 dates during the growing season of 2011.

Leaf gas exchange

Net photosynthesis rate (A_{net}), stomatal conductance (g_s) and light-induced dark respiration were measured on fully developed leaves during one campaign in the nursery, monthly during the growing season of 2010 (4 campaigns) and twice a month during the growing season of 2011 (7 campaigns) with a CO_2-H_2O infrared gas analyzer (IRGA; Licor 6400, LI-COR Inc., USA). The measurements were carried out at ambient temperature, air humidity and photon flux density (PPFD); CO_2 concentration was held constant at 400 ppm. Light-induced dark respiration was recorded using an opaque cuvette. Intrinsic water-use-efficiency ($iWUE$) was calculated as:

$$iWUE = \frac{A_{net}}{g_s}\ [\mu\mathrm{mol\,CO_2\ mol\,H_2O^{-1}}]$$

where A_{net} is the net CO_2 assimilation rate and g_s is the stomatal conductance for water vapor.

Destructive harvest

After each growing season, randomly selected beech saplings were harvested during the first two weeks of October by clipping the shoot and digging out the root system ($n_{nursery} = 20$ (only shoot), $n_{2009} = 27$, $n_{2010} = 36$, $n_{2011} = 81$ (60 complete, 21 only shoot).

Biometric and morphological shoot and root parameters

Basal area increment as a proxy for plant growth was deduced from tree ring widths analyzed by digital image evaluation (Sigmascan 5, Systat Software, USA) of stem thin-sections after final harvest in 2011. With this technique, plant growth could be back-traced for the years of experimental manipulation as well as for the nursery period. To provide comparability between the considered years and in order to compensate for allometric effects during plant development, the normalized basal increment relative to each preceding year was calculated (nrBAI, see chapter 2).
The foliage was scanned (600 dpi, 24 bit color depth) immediately after harvest to determine leaf area (SigmaScan 5, Systat Software Inc., USA) and average leaf color ('Leaf area', software, Hochschule Weihenstephan-Triesdorf, Germany) via graphical analysis; the latter, represented by its Hue-value [%], was regarded as a proxy for leaf senescence. Tiller lengths and ramification orders were recorded, then the mass of all shoot parts (stem (> 2 mm), twigs (< 2 mm), leaves and buds) was detected after oven-drying at 60 °C until constant weight was achieved.

Rooting depth was estimated during excavation by measuring the depth of the deepest coarse root (> 2 mm). The root compartment was extracted from the soil by washing with tab water and separated into live and dead fine roots (< 2 mm) and coarse roots. A representative subsample of the fine roots was scanned (400 dpi) and subjected to morphological analysis using the digital image evaluation software WinRHIZO (Regent Instruments Inc., Canada). The proportion of mycorrhizal root tips was estimated by visual examination. All roots were oven-dried at 60 °C and weighed.
The following parameters were calculated from the obtained data: Specific leaf area (SLA, $m^2\,kg^{-1}$), leaf area ratio (leaf area per total sapling biomass, LAR, $[m^2\,kg^{-1}]$), leaf mass fraction (leaf mass per total sapling biomass, LMF), mean bud weight [mg], twig ramification density [n m^{-1}], specific twig length (total twig length per twig weight, STL, [cm g^{-1}]), root/shoot biomass ratio (r/s ratio), root mass fraction (root biomass per total sapling biomass (RMF), average fine root diameter [mm], specific fine root length (fine root length per fine root biomass, SRL, [m g^{-1}]), specific root tip density (number of root tips per fine root biomass, SRTD, [10^3 g^{-1}]) and fine root ramification density (=SRTD/SRL, [n m^{-1}]).

Chemical plant parameters

Leaf samples (taken on August 24^{th} 2011; 10 leaves per sapling) and representative fine root samples (taken after final harvest) were analyzed for nutrient and non-structural carbohydrate (NSC) contents. Immediately after harvest, the plant material was shock-frosted with dry ice (-78 °C) and kept at -18 °C, freeze-dried and homogenized after milling.
Its molar C/N ratio was assessed by combustion analysis (varioEL, Elementar Analysensysteme GmbH, Germany). Mg, Ca, K, Mn and P [mol g^{-1} dry weight] were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES; Spectro Analytical Instruments, Germany) after digestion with HNO_3.
Analysis of non-structural carbohydrates in leaves and fine roots was performed according to Fleischmann et al. (2009): Water soluble sugars (stachyose, raffinose, sucrose, glucose, fructose) were separated by hot water extraction at 85 °C; starch was extracted from the remaining pellet after digestion with amylase and amyloglucosidase. Analysis was conducted using high performance liquid chromatography (HPLC) with a CARBOsep CHO-820 calcium column (Transgenomic, UK).
Natural abundance of ^{13}C was assessed in homogenized bud material collected immediately after final harvest using an isotopic ratio mass spectrometer (IRMS; delta S, Finnigan MAT, Germany) coupled to an elemental analyzer (NA 1108, CE Instruments, Italy).

Statistical analyses

In most cases, linear models were confirmed to be appropriate to describe the correlation between DSD, LD and the respective plant parameters. Bi-parametric regression analyses were performed after excluding collinearity of DSD and LD and log-transformation of DSD and LD. Interannual differences were tested via one-way-ANOVA and the Thamhane post-hoc test (SLA, A_{max}); for nrBAI, regression functions for the three years of experimental throughfall manipulation were tested for inequality of slopes via partialized regression (Armitage et al., 2002).

Morphological and chemical parameters (data derived from final harvest in 2011, Tab. 1.1) were subjected to incremental multivariate regression considering DSD and LD. To compensate allometric effects, total plant biomass was implemented into the regression model as nested effect. Three differently defined DSD were employed as explaining variables: (1) DSD during the growing season in which plants were harvested ('DSD_{2011}', 'current-year drought'), (2) DSD of the preceding growing season ('DSD_{2010}', 'preceding-year drought') and (3) average DSD of the three growing seasons of experimental throughfall manipulation representing the long-term average of drought ('$DSD_{2009-2011}$', 'average drought'). In reference to the results yielded for $\delta^{13}C$, the sensitivity of plant parameters to drought was ascribed to five response categories (Fig. 3.2 b) denoted with 'NR' = no response to DSD , 'I' = weak response to incipient drought , 'II' = weak response for high DSD, 'III' = sensitive response to incipient drought and 'IV' = sensitive response for high DSD. For detailed method description, see chapter 3.

1.3.2 Rhizotron experiment (study II)

Rhizotron set-up

24 one-year-old beech saplings were planted into rhizotrons (Fig. 4.1) filled with homogenized soil from the Bw horizon of the study site in spring 2009. The rhizotrons were designed to observe fine root growth and to measure CO_2 fluxes from the soil compartment and the shoot of the beech saplings. We installed nine additional control rhizotrons without beech saplings for assessment of the CO_2 flux from decomposition of soil organic matter. Each rhizotron was equipped with a FDR soil moisture sensor (ECH_2O 20, Decagon Devices, USA) that was vertically installed to integrate volumetric water content from 10 to 30 cm soil depth. The soil surface was covered with a sandy quartz layer with a thickness of 4 cm minimizing water loss by evaporation. The rhizotrons were placed on a cleared area (10 m^2) within the study site.

Soil water manipulation and drought quantification

In 2010, a translucent roof (height 1.5 m) was built above the rhizotrons to exclude natural throughfall and three treatments of soil water availability with 8 saplings each were established: no water limitation, moderate and severe water limitation corresponding to mean target soil water potentials of -0.03 MPa, -0.4 MPa and -1.0 MPa, respectively.
Sensor calibration was conducted in analogy to study I by regularly measuring Ψ_S at half-depth of the rhizotron soil. The time course of Ψ_S was modeled for each sapling based on the hourly logged sensor signal and the individual calibration spline in order to calculate DSD.

CO_2 flux measurements

Soil CO_2 efflux was measured on twelve dates using the dynamic closed chamber technique. The rhizotrons were sealed and CO_2 concentration in the rhizotron headspace (volume 0.95 l) was measured every 10 s over 4 min with an IRGA (LiCOR 820, Licor, USA). Soil CO_2 efflux was then calculated from the slope of the linear regression between CO_2 concentration and incubation time (Borken et al., 2006). Rhizosphere respiration was estimated from the difference in soil CO_2 efflux between planted rhizotrons and rhizotrons without saplings.
Net photosynthesis rate was also measured with a chamber (volume = 35 l) and a light source providing a constant photon flux density of 250 μmol m^{-2} s^{-1}. Net CO_2 uptake rate was recorded after a linear decrease of CO_2 concentration was observed and calculated in analogy to soil CO_2 efflux.

Stomatal conductance

As an additional indicator of drought stress, stomatal conductance of single leaves (n = 2 per plant) was measured 14, 26 and 64 days after the beginning of the drought treatment at ambient temperature around noon (LiCOR 6400, Licor, USA). The photon flux density during measurement was adjusted to 280 μmol m^{-2} s^{-1}.

Fine root production

Rhizotron side walls were photographed on eight dates during the growing season of 2010. Visible fine roots were analyzed by means of fine root length and diameter using the evaluation software WinRHIZO TRON (Regent Inc., Canada). By superposing the rhizotron images, fine root volume production and fine root length production were determined between every session.

Biometric plant parameters after harvest

The root system of the beech saplings was extracted by washing with tap water in October 2010 and separated into live and dead roots as well into fine (< 2 mm) and coarse roots. Morphology of live fine roots was assessed after scanning and

digital image evaluation (WinRHIZO, Regent Inc., Canada). SRL ($m\,g^{-1}$), relative fine root length distribution by fine root diameter [%], SRTD [g^{-1}] and fine root live/dead ratio were calculated after freeze-drying of the complete fine root system and detection of its dry mass.
Fresh leaves were scanned (600 dpi) immediately after harvest to determine foliage area (SigmaScan 5, Systat Software Inc., USA). All plant material was oven-dried at 40 °C until constant weight. Root/shoot biomass ratio was calculated from the dry mass of all roots and the complete shoot including foliage.

Non-structural carbohydrates in fine roots

A subsample of the freeze-dried and homogenized fine root material was ground and analyzed for its content of NSC (see 1.3.1).

Statistical analyses

Treatment differences were tested using ANOVA followed by the Tukey HSD test; in case of non-normally distributed data (Shapiro Wilk test, $p < 0.1$), the non-parametric Wilcoxon multiple comparisons test was conducted after Kruskal-Wallis-ANOVA. Influence of DSD on different plant parameters irrespective of the treatment collective was determined by linear regression.

1.3.3 Labeling experiment (study III)

Experimental set-up

The labeling experiment was carried out with beech saplings derived from the irrigation plots (study I). After two growing seasons of establishment in the stand without soil water limitation, 36 randomly chosen plants were transplanted into 20 l-containers including the rooted soil monolith in autumn 2010. Each container was equipped with a FDR soil moisture sensor (ECH_2O 20, Decagon Devices, USA), vertically installed at half-depth of the soil monolith. The pots were immediately re-inserted into the natural soil at the stand.

Soil water manipulation and drought quantification

A translucent roof construction (height 2.2 m) was built above the potted saplings in late June 2011 for throughfall exclusion. The saplings were randomly assigned to three drought treatments with unlimited soil water availability (control), moderate drought and severe drought, corresponding to mean target soil water potentials of -0.05 MPa, -0.6 MPa and -1.2 MPa, respectively. Assessment of $\Psi_S\,(t)$ and DSD as well as the adjustment of the respective drought level was conducted in analogy to study I and II.

First pulse label

In August 2011, the beech saplings were transported into a climate chamber and individually pulse labeled with ^{13}C-depleted CO_2 ($\delta\,^{13}$C = -47 ‰, DIN EN ISO 14175:C1, Westfalen AG, Germany) using transparent chambers (volume = 250 l) that also allowed for measurements of photosynthesis, light-adapted and dark-adapted shoot respiration and soil respiration (closed dynamic chamber technique) by an IRGA (LiCOR 820, Licor, USA). We labeled ten plants per treatment, two plants per treatment served as unlabeled controls.
^{13}C abundance in leaf respiration of every sapling was measured using gas bags. An exponential decay model was fitted to its time course in order to estimate mean residence time of recent assimilates (MRT) in leaf respiration. Label abundance was also recorded in soil respiration after sealing the container headspace. All gas samples were stored in 5 ml glass vials (Exetainer, Labco Limited, United Kingdom) and analyzed within 7 days for their isotopic signature (GVI-Isoprime, Elementar Analysensysteme GmbH, Germany).

Rewetting

The beech saplings were rewetted with deionized water six days after the first labeling, so that soil water potential ranged near field capacity in all pots.

Second pulse label

Five days after rewetting, a second labeling was carried out with $^{13}CO_2$ (99 atom % ^{13}C, Eurisotop, France). ^{13}C in leaf and soil respiration was monitored afterwards in analogy to the first labeling.

^{13}C mass balance

We calculated a ^{13}C mass balance for every beech sapling twelve days after the second labeling as follows:

$$m^{13}C_{uptake} = m^{13}C_{AR} + m^{13}C_{SR} + m^{13}C_{Bio} + m^{13}C_S$$

where $m^{13}C_{uptake}$ is the absolute amount of ^{13}C taken up by each plant during the labeling session, $m^{13}C_{AR}$ is the label-derived amount of ^{13}C emitted by aboveground respiration represented by leaf respiration, $m^{13}C_{SR}$ is the label-derived amount of ^{13}C emitted by soil respiration during the observed period, $m^{13}C_{Bio}$ and $m^{13}C_S$ is the label-derived amount of ^{13}C recovered in plant biomass and in the soil, respectively. The latter only refers to hot water soluble organic matter (SOM). Isotopic signatures were assessed by IRMS (gas samples: GVI-Isoprime, Elementar Analysensysteme GmbH, Germany; solid samples: see study I).

Statistical analyses

Treatment differences and influence of DSD on different plant parameters irrespective of the treatment collective was conducted in analogy to study II.

1.4 Synopsis and discussion of key findings

1.4.1 Drought quantification and DSD concept

Quantification of soil water potential

Estimated Ψ_S was compared to measured $\Psi_{L\,pd}$ (Fig. 1.3). If water homeostasis fully regenerates during nighttime, Ψ_L would theoretically equal Ψ_S (neglecting differences in gravitational and osmotic potential). In reality, limited hydraulic conductivity of the soil, especially within the rhizosphere, decelerates this process, so that the data points in figure 1.3 are expected to be located above the parity line. As this is mostly the case, it is concluded that the method of Ψ_S quantification was adequate and did not categorically overestimate drought. The intersection point of the parity line and the regression line may point to a disruption of the hydraulic link between roots and soil for Ψ_S < -0.4 MPa.
The quality of correlation between modeled Ψ_S and other instantaneous plant responses, e.g. photosynthesis and stomatal conductance (see below), further validates the approach of drought quantification.

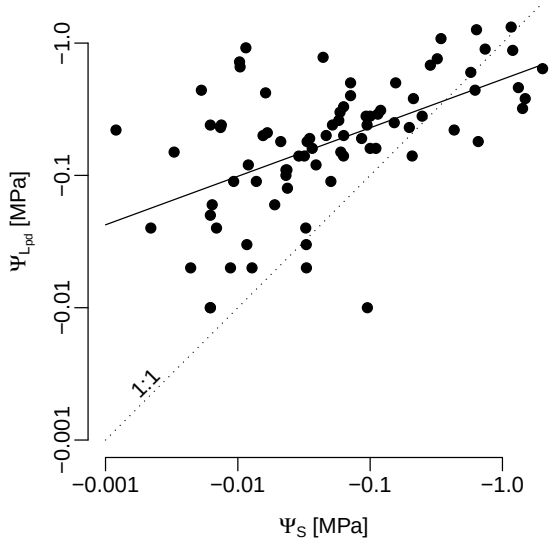

Figure 1.3: Pre-dawn leaf water potential and modeled soil water potential at the time of measurement (r^2 = 0.28, p < 0.0001). The dotted line indicates parity.

On the study site (study I), mean soil drought in the rooted space of the beech saplings increased every year with minimum soil water potentials of -0.9 MPa in 2009, -1.2 MPa in 2010 and -1.9 MPa in 2011 (Fig. 3.2). The drought range evoked by the treatments as reflected by DSD accounted for <1 - 37, <1 - 39 and

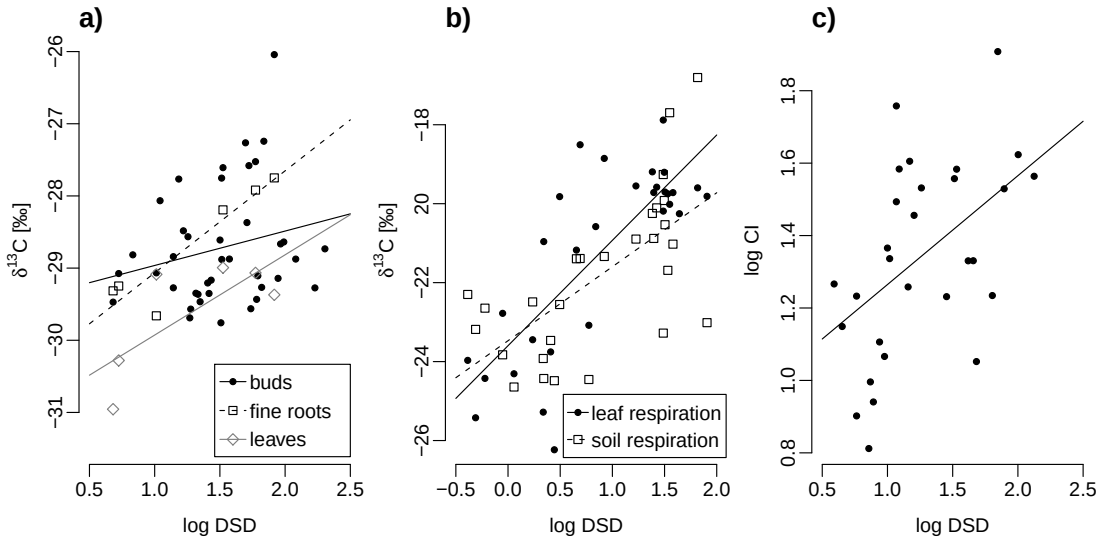

Figure 1.4: a) Correlation of DSD with natural abundance of ^{13}C in plant material: leaves ($r^2 = 0.43$, $p < 0.01$), buds (significant correlation with $r^2 = 0.23$ and $p < 0.05$ if a log DSD-threshold of 0.75 and the covariates *light dose* and *plant biomass* are considered (study I, see chapter 3) and fine roots ($r^2 = 0.82$, $p < 0.001$); **b)** Correlation of DSD with natural abundance of ^{13}C in respiratory C-fluxes: leaf respiration ($r^2 = 0.61$, $p < 0.0001$) and soil respiration ($r^2 = 0.42$, $p < 0.0001$) (study III, Fig. 5.2); **c)** Relationship between competition index and DSD, averaged over the three years of experimental throughfall manipulation ($r^2 = 0.27$, $p < 0.01$) (study I).

<1 - 133 MPa d for 2009, 2010 and 2011, respectively (Tab. 2.1). Soil water manipulation in study II resulted in a DSD gradient of [<1 ; 112] MPa d; DSD in study III ranged between <1 and 89 MPa d with minimum Ψ_S of <-1.5 MPa (treatment mean = -0.9 MPa) and -3.0 MPa (treatment mean = -1.4 MPa), respectively (Fig. 4.2, 5.1).

Physiological significance of DSD

Correlations of DSD with natural abundance of ^{13}C in different plant tissues or respiratory C fluxes (Fig. 1.4 a, b) were throughout positive and significant. This fact not only corroborates the assumption of drought influence on δ^{13}C by stomatal closure (Farquhar and Sharkey, 1982; Farquhar et al., 1989), it also proves the significance of DSD in reflecting long-term drought exposure (Fotelli et al., 2003, 2009), e.g. over one growing season: Especially δ^{13}C of plant tissues can represent mean environmental conditions during a certain period of time if recent assimilates were pre-eminently invested for tissue formation (cf. Hansen and Beck, 1994; Adams and Grierson, 2001). Thus, ^{13}C in buds was correlated to DSD calculated for the year of harvest (current-year drought) and not to long-term average of drought or preceding-year drought (Tab 3.2).

A considerable correlation was also identified for the competition index (Fig. 1.4 c) indicating that neighboring vegetation, besides its competition for light, decreases soil water availability of beech saplings. This is an important finding with consequences for statistical analyses concerning interaction between DSD and LD. In the context of DSD significance, the correlation of DSD to CI gives evidence of this parameter's time-dimensionality.

1.4.2 Plant responses to drought

Physiology and growth

As instantaneous drought responses, photosynthesis and stomatal conductance significantly decreased with decreasing Ψ_S (Fig. 2.5, 4.3) and DSD (Tab. 5.2). Minimum g_s of $< 0.04\,\mathrm{mol\,H_2O\,m^{-2}\,s^{-1}}$ (study II) indicated severe drought stress (Gallé and Feller, 2007). Adjustment towards increasing iWUE reflected the presence of a trade-off between carbon gain and water loss. Under field conditions (study I), PPFD was considered as covariate being positively correlated to A_{net} and g_s.
Photosynthesis became less sensitive to Ψ_S and g_s was generally lower in 2011 than in 2010, resulting in increased iWUE, also for unlimited soil water availability, in 2011 (Fig. 2.5). This shift is interpreted as an acclimation effect which coincides with a reduction of SLA as discussed later (cf. Fig. 2.4). The combined effect of DSD and LD on Ψ_S and g_s is plausible as nursery-derived saplings acclimated to reduced light exposition in the forest stand. Especially for the shade-tolerant species *F. sylvatica*, subsisting high-light acclimation from the nursery and its negative implications on net C gain under shade may have increased the drought effect in the first year upon transplantation (Björkman, 1981; Rodríguez-Calcerrada et al., 2010).
Within-plant assimilate transportation was hampered by drought with a significant correlation between MRT of recent assimilates in leaf respiration and DSD (Fig. 5.3 a and 5.5) and a delayed occurrence of label in soil respiration (Fig. 5.3 b) pointing to tissue dehydration and reduction in phloem loading due to reduced carbohydrate production (cf. Rühr et al., 2009). Rhizosphere respiration was reduced by drought by more than 50 % and cumulative rhizosphere respiration was significantly correlated to DSD (Fig. 4.4, 4.5) which indicated drought-induced C limitation of stressed saplings.

There was a strong depression of annual radial growth after sapling transplantation to the study site from more than 300 % in 2008 to less than 25 % in 2009 (Fig. 1.5). This planting shock is not only explained by damages to the root system and changes in nutritional status (Burdett, 1990; Grossnickle, 2005) but also by the concomitant necessity of drought and shade acclimation of the beech saplings in the forest stand. Throughout the three years following transplanta-

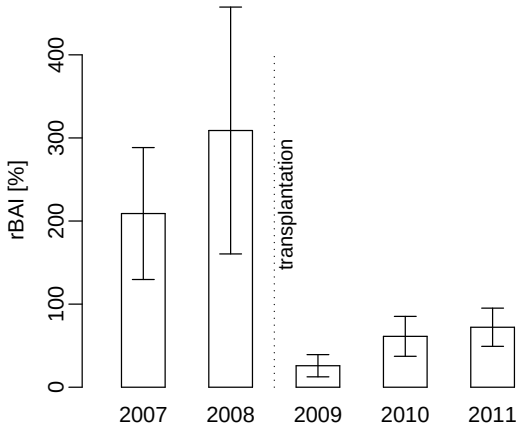

Figure 1.5: Relative basal increment during nursery and after transplantation to the study site, n = 81

tion, normalized relative basal area increment was negatively correlated to DSD and positively correlated to LD (Fig. 2.3) which is in accordance with the pattern observed for photosynthesis. Yet standardized slopes for DSD increased from -0.39 in 2009 to -0.23 in 2011 (Tab. 2.3), indicating that mechanisms of drought acclimation must gradually have facilitated plant growth.
After three years of experimental throughfall manipulation, shoot biomass was strongly reduced with increasing DSD, whereas root biomass hardly showed a response (study I, Fig. 2.2 a, b; Tab. 2.3). As a result, root/shoot biomass ratio was positively correlated to DSD (Fig. 2.2 c, 3.1) indicating a shift in resource allocation to the benefit of the belowground compartment (Kuster et al., 2012). After one growing season of drought treatment, a weak trend to increasing root/shoot biomass ratios with increasing DSD was also observed in three-year-old beech saplings (study II, Tab. 4.1) but absent in older saplings (study III, Tab. 5.2).
Fine root growth tended to increase under moderate drought and was hampered under severe drought (Fig. 4.6 a, b). This unimodal pattern resulted from the fact that increased fine root production is limited by C resources at increasing drought. When the soil is drying, root growth is also limited by physical resistance of the soil (Leuschner et al., 2001; Bengough et al., 2006). Fine root turnover could not directly be detected with the rhizotron technique as the identification of dead roots was only possible after destructive harvest. Fine root live/dead ratio, a proxy for fine root turnover, was negatively correlated to DSD (Tab. 4.1) which might indicate an acceleration of fine root turnover; however, necromass decomposition is likely to be reduced under drought, rendering this conclusion vague (Godbold et al., 2003; Leuschner et al., 2004; Gaul et al., 2008; Persson and Stadenberg, 2009; Zang et al., 2011b).

Morphology

Decreasing SLA and LAR (Tab. 3.1) indicated a trend towards increased sclerophylly and might partly be responsible for increasing iWUE after repeated summer drought. Interestingly, both parameters were correlated to preceding-year drought (DSD_{2010}) and exhibited a sensitive response already upon incipient drought (both parameters were ascribed to response category III) which supports the assumption that leaf size is not only governed by cell turgor during expansion but also is predetermined by earlier drought events (Löf and Welander, 2000). A further key finding concerning shoot morphology was a negative correlation of the Hue-value of collected leaves to current-year drought (category IV, Tab. 3.1). Leaf senescence is a common drought response likely being associated with earlier leaf shedding (Chaves et al., 2003) that has been shown to take effect in the range of moderate to severe drought. Incremental multivariate regression demonstrated that leaf senescence of European beech saplings also correlated to $DSD_{2009-2011}$ (category III) suggesting that there is a long-term drought effect generally leading to shorter leaf life spans.

The most marked morphological response to drought stress was identified for rooting depth (Fig. 2.6 a; Tab. 3.1) which was significantly correlated with $DSD_{2010,}$, DSD_{2011} as well as $DSD_{2009-2011}$; analysis revealed a sensitive response of this parameter already upon incipient drought (all response category III). This finding corroborates optimal partitioning theory (Bloom, 1985) as deeper rooting promotes water uptake by exploitation of moister soil layers. Further, a pronounced morphological plasticity of the belowground compartment of *Fagus sylvatica* is evidenced.

Fine root morphology, in general, responded less distinctly and exhibited an immense variability: There was no significant response of specific root length in any experiment. After three years of drought treatment, average fine root diameter decreased with increasing drought (Fig. 2.6) and exhibited a weak yet significant response upon incipient drought (category I for DSD_{2011} and $DSD_{2009-2011}$, Tab. 3.1). A similar trend was also demonstrated in the rhizotron experiment as relative diameter class length of roots < 0.2 mm was significantly increased in the drought treatment (Fig. 4.7). Specific root tip density tended to increase with increasing DSD already after one growing season (Fig. 1.6 b; Tab. 4.1); after three years of treatment, a significant response of SRTD became apparent (Fig. 1.6 b; Tab. 3.1).

Also for fine root morphological parameters, the combination of drought acclimation and allometric adjustment is relevant as exemplified for SRL and SRTD (Fig. 1.6): Trends to increasing SRL and SRTD as strategies to increase water uptake and soil exploitation were observed both with increasing drought exposure as well as from year to year. The former (=acclimation) is visualized by the non-null

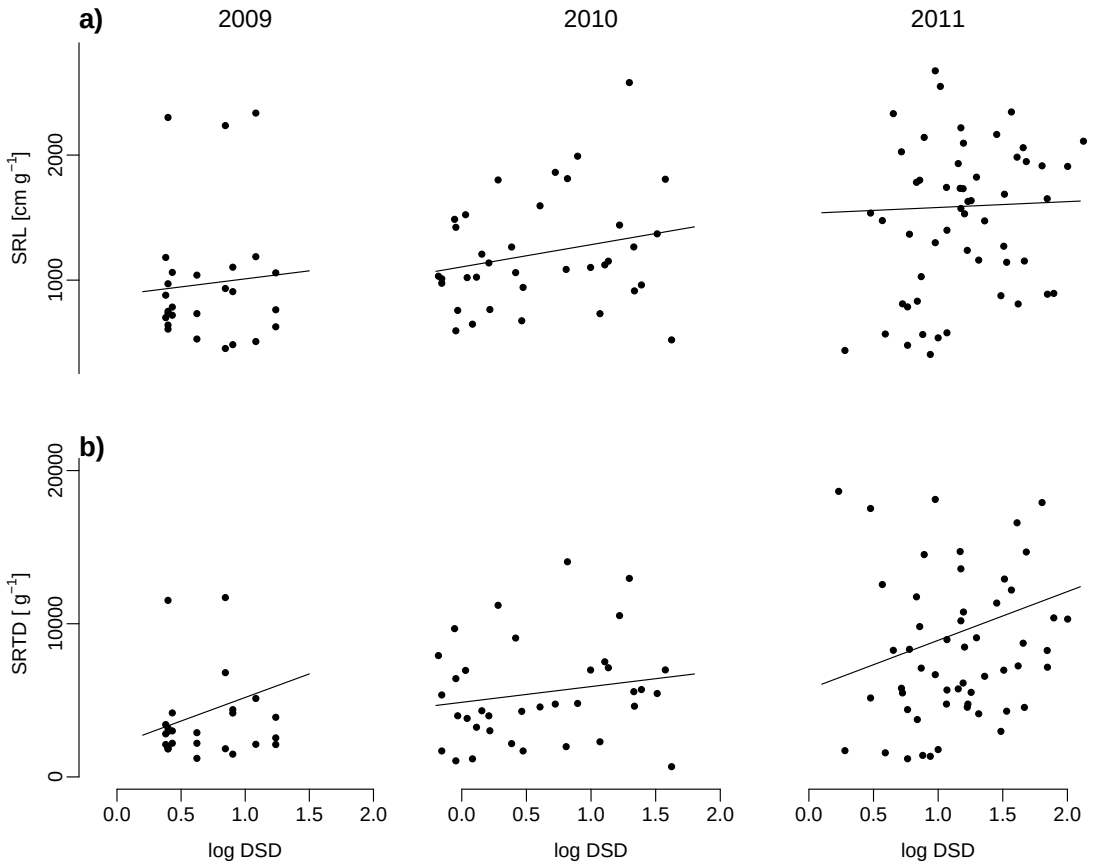

Figure 1.6: Specific root length (**a**) and specific root tip density (**b**) for the three years of experimental throughfall manipulation correlated to DSD of the corresponding year ($n_{2009} = 27$, $n_{2010} = 36$, $n_{2011} = 59$).

slope of the regression lines in each year, while the latter (=ontogeny) is reflected by different - in this case: increasing - group means of consecutive years.

Chemical parameters

Results of NSC analyses differed between study I and study II threatening a general statement (Tab. 3.3, 4.1). Whereas increased fructose concentration in fine roots was interpreted as an indicator of osmoregulation in two-year-old saplings from the rhizotrons (Kameli and Lösel, 1993), no response of fructose concentration was observed in fine roots or in leaves of the older saplings from study I (Tab. 3.3). The latter exhibited increased sucrose concentrations in leaves (category IV), but the correlation was only significant for $DSD_{2009-2011}$. Thus, osmoregulation processes in beech saplings are not certainly evidenced. The rhizotron-saplings exhibited a non-significant trend towards increased total NSC and starch reserves in fine roots (not shown), whereas, in study I, a strong starch depletion in leaves under severe drought (category IV for DSD_{2010} and DSD_{2011}, Tab. 3.3) was attributed to enhanced respiratory C demand as total NSC reserves continu-

ously decreased in fine roots with preceding-year drought (category III, Tab. 3.3). The correlation was absent for current-year drought pointing to a certain inertia of the NSC pools. These opposed findings are explained by differing sapling age and differently advanced drought acclimation in the two experiments. In conclusion, we concur with Ryan (2011) who stated that NSC status, due to complex interferences, might not necessarily be of significance as a drought indicator.

Differences in nutrient partitioning in roots and leaves upon drought became apparent e.g. for Mg concentration which sensitively decreased in fine roots whereas, in leaves, it was relatively constant over a broad range of drought stress (Tab. 3.2). This might be the result of control mechanisms that aim at maintaining photosynthesis as long as possible (Peuke and Rennenberg, 2004, 2011). Molar C/N ratios increased both in leaves and fine roots with increasing drought. The former was correlated to DSD_{2011} (category III) while the latter was correlated to $DSD_{2009-2011}$ (category IV) which is explained by longer life spans of fine roots compared to that of leaves: leaves are assumed to reflect reduced N uptake during drought whereas root chemistry might rather be representative for the long-term average. Similarly, P concentrations decreased in leaves (category I for DSD_{2010}) and stayed unchanged in fine roots (Tab. 3.2).
Decreasing concentrations of Ca and K are explained by immobilization of these nutrients in dry soil (Tab. 3.2). No drought-induced changes of K concentration were found in leaves; possibly, reduced K availability masked concurrent osmoregulation by enhanced release of K (Maathuis, 2009; Peuke and Rennenberg, 2011).

1.4.3 Recovery after drought

After rewetting of the soil, photosynthesis recovered to initial level within five days (Tab. 5.2); also in the rhizotron experiment, water input in August of 2010 after heavy rain events led to a fast recovery of A_{net}, suggesting that photosynthesis decrease during drought was rather due to stomatal than to non-stomatal limitation (Gallé and Feller, 2007). Furthermore, even after severe drought stress, mean residence time of recent assimilates five days after rewetting did not exhibit a correlation to DSD any more; there were also no differences in label occurrence in soil respiration (Fig. 5.3-5.5; Tab. 5.2) indicating a fast recovery of processes involved in assimilate transportation. However, ^{13}C mass balance revealed differences in C partitioning as an increased proportion of recent assimilates was invested in aboveground respiration of formerly drought-stressed saplings (16 % of label-derived ^{13}C in the control vs. 24 % under severe drought, Fig. 5.6; Tab. 5.2) which might be associated with repair processes e.g. of embolism or the photosynthetic apparatus (Bréda et al., 2006; McDowell et al., 2008). A more distinct after-effect of drought became apparent in the partition-

ing of ^{13}C in plant biomass (significant negative correlation to DSD) indicating decreasing C reserves and/or tissue growth with increasing drought stress. Also the proportion of label-derived ^{13}C in the root compartment was smaller (45%) in formerly drought-stressed beech saplings compared to non-stressed saplings (64%, Fig. 5.7; Tab. 5.2). In conclusion, the effect of drought stress in the different treatments mostly vanished on a time scale of days. Persistent drought effects on C partitioning, albeit significant, were not fundamental.

1.5 Conclusions

Even under severe drought stress with perceptible consequences e.g. for physiological processes and C fluxes, planted *Fagus sylvatica* saplings exhibited a considerable resilience against soil drought given that no drought-induced mortality has been observed. Drought resilience further increased with repeated summer drought from year to year, pointing to effective acclimation processes, especially of morphological traits. This plasticity is assumed to ensure the fitness of European beech saplings under drier climatic conditions.

Drought responses of beech saplings interfered with light conditions which may strongly vary in open forests. Especially for nursery-derived saplings, previous light acclimation modifies drought responses and has to be considered in silvicultural practice. In the nursery, drought hardening together with acclimation to different light regimes might therefore be advantageous for seedling performance under adverse conditions after transplantation (van den Driessche, 1991; Vilagrosa et al., 2003; Villar-Salvador et al., 2004; Guarnaschelli et al., 2006, 2012).

Owing to the DSD approach, drought responses were described by dose-response-functions and ascribed to response categories. This approach of drought quantification consorts with monotonically evolving drought as simulated in the presented experiments. It does not consider drought cycles or the date of drought events during the growing season. Such drought scenarios are believed to cause more complex responses (Kozlowski, 1992; Guo et al., 2010; Guarnaschelli et al., 2012; Niinemets, 2010). However, by its scalability, DSD provides a reference for future studies and is considered as a further step towards an integrated understanding of soil-plant-interaction.

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Manuscripts

2 Growth of juvenile beech (*Fagus sylvatica* L.) upon transplant into a wind-opened spruce stand of heterogeneous light and water conditions

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2.1 Abstract

Climate models predict increasing frequency and intensity of summer drought events for Central Europe. In a field experiment, we investigated the response of young beech (*Fagus sylvatica* L.) to extreme and repeated summer drought and the modulation of drought response patterns along the natural gradient of light availability at the study site.
In autumn 2008, two-year-old, nursery derived beech - as used for forest conversion practices - was planted under a Norway spruce stand primarily opened through winter storm. Precipitation was manipulated in the growing seasons of 2009 through 2011, inducing a pronounced gradient of water availability. Individual drought-stress doses (DSD) and light doses (LD) were calculated for each beech sapling during the three growing seasons.
Plant growth, CO_2-assimilation rate and stomatal conductance were reduced with increasing drought stress, but facilitated by increasing light availability. Progressive acclimation to water and light limitation during the three years of the experiment led to a decreased drought and shade sensitivity of diameter growth. Water-use efficiency, root/shoot ratio and rooting depth, were increased with decreasing water availability. Mean fine root diameter correlated negatively with both DSD and LD. Proceeding low-light acclimation was indicated by progressively increasing specific leaf area and reduced leaf dark-respiration.
We conclude that nursery conditions induced a high-light acclimation of the beech saplings, exacerbating productivity decline under co-occurring water and light limitation.

2.2 Introduction

Research on climate change predicts rising air temperature along with increasing frequency and intensity of summer drought for Central Europe during the upcoming decades (IPCC, 2007). Under such perspective, conversion of pure plantations of *Picea abies* [L.] KARST. or *Pinus sylvestris* L., when cultivated outside their areas of natural ecological distribution, to mixed broadleaf-coniferous forests is crucial towards increasing forest stability (Bolte et al., 2007; Kazda and Pichler, 1998). Exemplifying the region of Bavaria in Germany, 260,000 hectares of coniferous forest cultures are to be subjected to conversion (Bayer. StMLF, 2011) to reduce ecological and economical risks (Knoke et al., 2008) by abiotic and biotic agents (Albrecht et al., 2010; Rouault et al., 2006; Schütz et al., 2006). Because of a wide ecological amplitude and potential dominance in natural sub-mountainous forest ecosystems of Central Europe (Ellenberg, 1996), European beech (*Fagus sylvatica* L.) is attractive for employment in forest conversion. However, drought is regarded as one major factor that limits the distribution of beech (Aranda et al., 1996; Backes and Leuschner, 2000;

Horvat et al., 1974), in particular when considering the drought sensitivity of juvenile beech trees (Löf et al., 2005; Löf and Welander, 2000; van Hees, 1997) and their risk of becoming outcompeted by early-successional grass and shrub species (Coll et al., 2004; Fotelli et al., 2002).
During the last decade, several studies with seedlings and saplings have provided contrasting evidence about the drought sensitivity of beech, resulting in controversial conclusions about the species future under climate change (Ammer et al., 2005; Gessler et al., 2006; Rennenberg et al., 2006). To our knowledge, all such studies had in common either to be based on naturally regenerated or sown plant material, or that the first years upon transplantation had been disregarded, supposedly to prevent evidential artifacts and variation. Conversely, transplanted plant material often suffers from root injury and insufficient root-soil connectivity, leading to deficits in water and nutrient uptake and subsequently reduced growth performance (Burdett, 1990; Grossnickle, 2005). Eventually, reduction in foliage area and altered whole-plant carbon allocation may occur (Struve and Joly, 1992). Another factor that affects plant material upon transplantation is the abrupt change in light exposure between nursery conditions and plantation sites. In practice, plant material is grown under optimum growth conditions, as guidelines for production (e.g. Schlegel, 2009) favor size development and vigor of plants. Consequently, nursery plants are typically grown under open site conditions and hence are acclimated to high insolation, whereas below-canopy conditions are characterized by spatio-temporally varying degrees of shading. Predisposition upon previous light acclimation can determine plant morphology and physiology (Eschrich et al., 1989). Relative to shade-grown plants, acclimation to high insolation results in plants with higher maximum CO_2-assimilation rates (A_{max}) and higher water-use efficiency (WUE) of photosynthesis at light saturation. Such latter differentiation, in addition, typically includes low foliage and specific leaf area (SLA), high leaf dark respiration rate (R), high light compensation points and photosynthetic light saturation at high irradiance. Hence, carry-over effects due to high-light acclimation are disadvantageous under low light conditions and likely to affect plant productivity and stress tolerance upon transplant. As high carbon gain requires high water uptake, regeneration upon transplantation may be viewed as a *positive cycle of root growth and photosynthesis* (Burdett, 1990), with enhanced water uptake upon root regeneration enabling photosynthesis to provide more carbon for further root growth. Hence, such a positive feedback may be disrupted in nursery derived plant material, if abruptly confronted with water and light limitation, exacerbating the transplantation shock. Root morphology under such conditions appears to be crucial (Čater and Simončič, 2010; Curt et al., 2005; Meier and Leuschner, 2008). While the response of shade-acclimated juvenile beech to an increase in light exposure and to co-occurring water limitation has been investigated in previous

studies (Aranda et al., 2001a,b, 2004; Fotelli et al., 2003; Löf et al., 2007), consequences of decreased light availability in combination with water limitation upon transplantation remain unclear. Taking into account that transplanting of nursery material is a common practice in forest conversion, the capacity of transplanted beech saplings for coping with water and light limitation is crucial during stand establishment below a canopy of old trees. Drought-related risks may be increased, in particular, under conditions of climate change. The identification of critical factors and a better understanding of their effect on plant performance upon transplant to the forest site, are decisive for refining methods for evaluation of seedling quality (Duryea, 1985).
The present study aimed, therefore, to clarify the responsiveness and acclimation ability of recently transplanted beech saplings to variable shading by a partially-opened spruce canopy at the restoration site in combination with experimentally repeated summer drought throughout a three-year study period. The hypotheses were tested that (i) co-occurrence of water and light limitation exacerbates reduction of growth, (ii) light limitation hinders the adjustment of the root/shoot biomass ratio and of root morphology to drought conditions and that (iii) water and light limitation intensify the planting shock upon transplantation.

2.3 Materials and Methods

Study site

The study was conducted in a 140-year-old Norway spruce stand (*Picea abies* (L.) H. Karst), located in the Fichtelgebirge in North-East Bavaria (50° 8' N, 11° 52' E, 770 m a. s. l.). Prior to the experiment, the stand was thinned through winter storm (Jan. 2007) to a stem density of < 150 per ha. The long-term average of the annual sum of precipitation (P_{annual}) in the study area was 1163 mm with an average of 574.3 mm during the growing season (May - Oct). The mean annual air temperature (T_{air}) was 5.3 °C, with a growing-season average of 11.2 °C. The mean annual relative air humidity (RH_{air}) was 82 %, with 78 % during the growing season (Gerstberger et al., 2004). Except for 2010, a year with extraordinarily high precipitation in August (264 mm), the study period was characterized by lower mean precipitation, higher mean temperature and higher relative air humidity (Instuments for climate monitoring at the study site: P_{annual}: OMC-212, OBSERVATOR instruments B.V., Netherlands; T_{air} and RH_{air}: HMP45A; Vaisala Oyj, Finland) as compared with the long-term average across 1971-2000 (Table 2.1). The soil derived from deeply weathered granites of Ordovician age, being classified as a Haplic Podsol with the organic layer corresponding to a moder humus form of a thickness of 7 - 10 cm (Schulze et al., 2009).

Table 2.1: Annual values of precipitation, air temperature and relative air humidity (both latter values measured 3 meters above ground) and values of the growing season (May - Oct) shown in brackets each. Data across 1971 - 2000 (Gerstberger et al., 2004). Data across 2009 - 2011 from climate monitoring at the study site. Annual amounts of water excluded (by roofs) and irrigated (by sprinkler) and ranges (given as intervals) of measured water potentials and drought stress doses in the growing seasons (May - Oct) of 2009 through 2011. Annual global radiation and values of the growing season (Apr - Oct) shown in brackets each and the gradient of LD and PACL during growing season.

	1971-2000	**2009**	**2010**	**2011**
P_{annual} [mm]	1162 (574)	972 (491)	1084 (794)	885 (531)
Manipulation of P_{annual} [mm]				
excluded	-	-142	-426	-304
irrigated	-	+20	+25	+50
Overall range of Ψ_{soil} [MPa]		[-0.9;-0.01]	[-1.2;-0.01]	[-1.9;-0.02]
Overall range of DSD [MPa*day]		[1;37]	[1;39]	[2;133]
Mean annual T_{air} [°C]	5.3 (11.2)	6.6 (12.3)	5.2 (11.6)	5.8 (12.6)
Mean annual RH_{air} [%]	82 (78)	86 (83)	92 (89)	86 (82)
Mean G [$W\,m^{-2}$]		199 (173)	117 (158)	133 (183)
Overall range of LD [$MJ\,m^{-2}$]		[324;971]	[300;899]	[341;1022]
Overall range of PACL [%]		[9;33]	[9;33]	[9;33]

Plant material

In autumn 2008, the study area was replanted with two-year-old beech trees (*Fagus sylvatica* L.) of a local provenance from north-eastern Bavaria (origin code: 81012). The plant material was provided by a local nursery (Bayerische Staatsforsten, Bindlach/Bayreuth, Germany). The nursery is located about 30 km (49° 58' N, 11° 34' E) from the study site at about 445 m a.s.l. In the nursery, plants were seeded in spring 2007 and harvested in autumn 2008 just before transplantation to the forest site. During growth in the nursery, the seedlings were neither transplanted nor undercut. In 2008, the plants were harvested excavating the root system down to a depth of 27 cm. In the nursery, seedlings had been exposed to full sunlight and watered to prevent soil drought. According to the common silvicultural practice, the bare-rooted plants were set into the mineral soil horizon at about 20 cm depth choosing a density of 2500 plants per hectare.

Light conditions and light dose

Upon wind-throw in 2007, the remaining stand canopy was patchy, causing heterogeneous light conditions on the forest floor. The individual light regime of the beech plants was assessed in summer 2011 by hemispherical photography (Camera equipment: WinSCANOPY DSLR 2005a system, Regent Instruments Inc., Canada) at the top height of saplings. The light regime was assessed based on the course of the site-specific global radiation (G) (CM14; Kipp&Zonen, Netherlands) above the canopy (data from local climate measurement tower) during the growing seasons in 2009, 2010 and 2011 and the hemispherical photo of the considered plant individual (Software for image analysis: WinSCANOPY v. 2005, Regent Instruments Inc., Canada). The individual light dose (LD) during the growing season was calculated as:

$$LD = \int R_{below\,canopy}\,(t)\;dt\;\;[MJ\,m^{-2}]$$

with $R_{below\,canopy}$ as the daily sum of direct and diffusive radiation reaching the forest floor at the regarded time t. The gradient of light availability given in percent above canopy light (PACL) ranged from moderate shade (33 PACL) to deep shade (9 PACL) conditions (Table 2.1).

Manipulation of precipitation and plant available soil water

During the growing seasons of 2009 through 2011, precipitation on 6 (2×3) plots of 400 m^2 was manipulated to reinforce the natural heterogeneity of water availability as follows: (i) Periodic rainfall exclusion (RE): wooden roof constructions (height: 2.5 - 3 m) were closed with transparent roof panels for 8 weeks in 2009 (June 15^{th} through August 15^{th}) and for 12 weeks each in 2010 and 2011 (June 10^{th} through September 10^{th}, each). The perimeter of the plots was trenched to 30 cm soil depth, inserting a waterproof plastic membrane to avoid lateral water flow. (ii) Non-limiting water availability via additional irrigation (I) with deionized water using an automatic sprinkler system controlled via tensiometers that were installed in the mineral soil horizon. Irrigation started whenever soil water potential fell below a threshold of -0.015 MPa. On three additional plots no manipulation (NM) of the natural water availability has been conducted (see Table 2.1 for amounts of excluded and irrigated precipitation).

Volumetric soil water content in the rooting zone (down to 30 cm of mineral soil depth) of each study plant ($n_{2009-2011} = 135$ for all plots in total) was hourly measured (FDR probe 10HS, Decagon Devices Inc., USA) and recorded (DL2e data logger, Delta-T Devices Ltd., UK). The signal of the employed type of FDR sensors integrates over a sensitive volume of approximately 1160 cm^3 (Cobos, 2008). During the growing seasons of 2009 through 2011, weekly measurements of soil water potential in the sensors detection range were performed with a portable

tensiometer (T5, UMS GmbH, Germany: for soil water potentials > -0.3 MPa) and a dew point potentiometer (WP4, Decagon Devices Inc., USA, for soil water potentials < -0.3 MPa). The FDR sensor signals were correlated with the corresponding soil water potentials (Ψ_{soil}). A spline was fitted to each dataset and used for modeling the time course of soil water potential in the rooting zone of the individual plants.

Validation of this proxy for plant available soil water via correlation of modeled soil water potentials with leaf water potentials potentials (measured in three campaigns in 2011: Jun. 22^{th}, Jul 27^{th} and Sept. 09^{th}; n = 20 each), assessed at predawn using a Scholander pressure chamber (Plant Water Status Console 3005 series, Soilmoisture Equipment Corp., USA), provided highly significant results (range of soil water potentials: [-1.9; -0.012] MPa; Spearman-Rho: coeff. = 0.67, $p < 0.0001$).

The cumulated soil water potential during the growing seasons (Apr. - Oct.) was defined as drought stress dose (DSD):

$$DSD = \int \Psi_{soil}(t)\ dt\ \ [MPa * day]$$

Due to the effect of pronounced small-scale heterogeneity in soil conditions and competing vegetation on soil water availability, precipitation manipulation did not induce homogeneous and separable treatments, but enforced the dry end of the gradient in water availability (intervals of DSD in 2009: I-plots [1.2; 3.6] MPa*day; NM-plots [2.5; 12.1] MPa*day; RE-plots [0.9; 37] MPa*day). As the entire gradient became established in the rainfall exclusion plots, only here assessments were focused in 2011.
Due to the shorter drought treatment of 8 weeks in 2009 less precipitation was excluded than in the subsequent years. Heavy rainfall, low temperatures and high relative air humidity in August 2010 impeded further drying of the soil. Hence, despite the longer drought treatment in 2010, only slightly lower soil water potentials and DSDs were reached than in 2009. 2011 yielded lowest soil water potentials and highest DSD during the entire 3-year study (Table 2.1).

Plant growth and morphology

At the end of each growing season, randomly selected plants ($n_{nursery} = 20$; $n_{2009} = 18$ with 2 plants from each plot; $n_{2010} = 36$ with 4 plants from each plot; $n_{2011} = 60$; with 20 plants from each rainfall exclusion plot) were harvested to asses specific leaf area (SLA) and tree ring width. Rooting depth, mean fine root diameter, specific root length (SRL) and plant biomass was assessed in 2011 on a subsample of 30 plants.
By excavating the root system, rooting depth (deepest root with a diameter > 2 mm) was determined. After harvest, roots were washed from soil particles

and the fine root system (diameter < 2 mm) was scanned at 400 dpi for morphological analysis using digital image evaluation software (WinRHIZO, Regent Instruments Inc., Canada). The fresh leaves were scanned (600 dpi, 24 bit color depth) immediately after harvest to determine the projected leaf area (SigmaScan 5, Systat Software Inc., USA). Annual stem diameter increment was determined after harvest in 2011 via graphical analysis (SigmaScan 5, Systat Software Inc., USA) on fresh cross-sectional cuts from the root collar, so that growth was backtraced to the time in the nursery.
For each study year, the individual relative basal area increment (rBAI) was correlated with DSD and LD. A linear three-dimensional regression model was found to be appropriate for describing the respective relationships. Modeled values derived from the calculated regression functions, were employed to analyze the effect of extreme combinations of DSD and LD (combinations of low/high logDSD: 0.1/1.6 MPa*day, low/high logLD: 2.4/3.0 MJ m^{-2}) on rBAI in the course of the subsequent years of 2009 through 2011. To focus on the effect of DSD and LD on diameter growth, different ranges of rBAI within the three years of the experiment were excluded via mean value normalization of rBAI for the respective year. Slopes of the regressions for nrBAI in correlation with DSD and LD served as a proxy for the effect strength of water and light availability on diameter growth. Changes of the regression slopes were analyzed for the subsequent years of 2009 through 2011. Biomass of the different plant compartments was determined on oven dried (65 °C until constant dry weight) plant material. Formulas for calculation of the plant parameters c.f. Table 2.2.

Leaf gas exchange

Leaf gas exchange was measured during one campaign in the nursery, monthly during the growing season of 2010 (4 campaigns) and twice a month during the growing season of 2011 (7 campaigns). Measurements were conducted on fully developed leaves. A portable CO_2-H_2O infrared gas analyzer (Licor 6400, LI-COR Inc., USA) was used, equipped with a cuvette providing red-blue light (6400-02B LED light source, LI-COR Inc., USA). Measurements were conducted during 10:00 to 15:00 CET each and under ambient temperature (nursery: 26.3 °C ±0.96 SD; study site 2010: 23.9 °C ±3.12 SD; study site 2011: 23.2 °C ±2.94 SD) and air humidity (nursery: 48.4 % ±3.25 SD; study site 2010: 61.7 % ±8.57 SD; study site 2011: 55.4 % ±6.24 SD). The corresponding vapor pressure deficit of the air during the measurements was 1.71 (±0.21 SD) kPa in the nursery, 1.12 (±0.43 SD) kPa in 2010, and 1.18 (±0.24 SD) kPa in 2011. CO_2 concentration was controlled to 400 ppm. For each measure campaign, plants were randomly selected with $N = 10$ in the nursery and $N_{2010} = 22$ and $N_{2011} = 46$ during the experiment. Prior to each measurement the ambient photosynthetic active photon flux density (PPFD) was determined with a photon flux sensor at the location of the sample leaf to ob-

tain the subsequent target PPFD level of the LED light source in the cuvette. Gas exchange was recorded upon reaching steady state. This procedure allowed instantaneous measurements at simulated ambient PPFD, while simultaneously avoiding changes in light availability during the measurement. Light-induced dark respiration was recorded during daytime, by inserting the leaf into the darkened cuvette. Intrinsic water-use-efficiency (iWUE) was calculated employing the measured values of assimilation rate and stomatal conductance (g_s) (Table 2.2).

Table 2.2: Formulas used for calculation of plant parameters. $rBAI_{i,a}$: relative basal area increment of plant i in year a, BAIi,a: basal area increment of plant i in year a, $BA_{i,a-1}$: basal area of plant i in previous year a 1, $nrBAI_{i,a}$: mean value normalized rBAI of plant i in year a, $\varnothing rBAI_a$: arithmetic mean of rBAI of all plants in the sample in year a, SLA: specific leaf area, $A_{leaf\,fresh}$: leaf area of the fresh leaves, $M_{leaf\,dry}$: dry mass of the leaves, SRL: specific fine root length, $L_{root\,fresh}$: total length of the fresh fine roots, $M_{root\,dry}$: dry mass of the fine roots, iWUE: intrinsic water-use-efficiency, A_{net}: net CO_2 assimilation rate, g_s: stomatal conductance for water vapor.

parameter	**formula**
relative basal area increment	$rBAI_{i,a} = BAI_{i,a} / BA_{i,a-1} * 100\,[\%]$
mean value normalized rBAI	$nrBAI_{i,a} = rBAI_{i,a} / \varnothing rBAI_a$
specific leaf area	$SLA = A_{leaf\,fresh} / M_{leaf\,dry} \quad [m^{-2}\,kg^{-1}]$
specific root length	$SRL = L_{root\,fresh} / M_{root\,dry} \quad [cm\,g^{-1}]$
instrinsic water-use-efficiency	$iWUE = A_{net} / g_s\,[\mu mol\,(CO_2)\,mol\,(H_2O)^{-1}]$

Statistical analyses

Spline fittings of non-linear relationships between measured soil water potentials and corresponding signals of the FDR sensors were calculated using R 2.13.0 (R Development Core Team, 2011). Statistical tests were conducted using PASW Statistics 18 (Release Version 18.0.0; IBM SPSS Inc., 2009). For SLA, A_{max} and R interannual differences were tested via one-way ANOVA. Due to inhomogeneous variances of the tested groups (Levene statistic), Thamhane as non-parametric post-hoc test was used. Except for gas-exchange data, tests of linearity (linearity of studentized excluded residuals) confirmed linear models to be appropriate to describe correlations between the analyzed plant parameters and log-transformed DSD and LD. Tests proved normal distribution (Shapiro-Wilk test) and constancy of variance of correlated parameters. After proving independency of DSD and LD (non-significant results for Pearson correlation), correlations were tested via bi-parametric linear regression. Interaction-effects of DSD and LD on plant parameters were tested via the PASW plug-in MODPROBE which is described in Hayes and Matthes (2009). The regression functions for nrBAI vs. DSD and

LD in the subsequent years of 2009 through 2011 were tested for inequality of slopes (c.f. ARMITAGE et al., 2002) via partialized regressions for DSD and LD. Non-linear regressions for gas-exchange data were computed using TableCurve3D (Release Version 4.0, Systat Software Inc., 2002). Linear regressions were calculated using SigmaPlot 12 (Release Version 12.0, Systat Software Inc., 2011).

2.4 Results

In general, all measured parameters showed a large variance, leading to relatively low r^2 of the calculated regressions. Nevertheless, statistically significant regression slopes in dependency of DSD and LD were obtained for the majority of the analyzed plant parameters. Interaction analysis via MODPROBE and residual analysis showed no significant interaction-effect (synergism or antagonism) of DSD and LD on any of the regarded plant parameters. However, an additive effect of DSD and LD on plant response was observed.

Biomass and diameter growth

Average whole-plant biomass for all plants increased from 37 (±14.5 SD) g in autumn 2008 to 143 (±72.6 SD) g in autumn 2011. In parallel, aboveground biomass development was about two-fold higher than belowground, leading to decreasing R/S-ratios. Average stem diameter increased from 6.9 (±1.1 SD) mm in autumn 2008 to 12.8 (±2.3 SD) mm in autumn 2011. Optimum growth conditions in the nursery (2008) had led to high average relative basal area increment (rBAI) of 309 (±148 SD) % which was reduced to 25 (±13 SD) % upon transplanting in 2009. After three years of establishment at the forest site, rBAI had recovered to 71 (±23 SD) % in 2011. In 2009, limitations by drought (DSD of 1.6 MPa*day = high DSD) in combination with low light (LD of 2.4 MJ m^{-2} = low LD; Fig. 2.1) almost caused diameter growth to cease (i.e. rBAI < 2% of the previous-year level in the nursery). Conversely, enhanced water and light supply (DSD of 0.1 Mpa*day = low DSD and LD of 3.0 MJ m^{-2} = high LD) caused an rBAI of 13 % of the level in 2008. The combinations high DSD × low LD and low DSD × high LD led to intermediate rBAI levels of 7 % and 8 % of rBAI in 2008 respectively. During the consecutive years, plants recovered from the planting shock. In 2010 and 2011, plants with enhanced supply of water and light recovered to 32 % of the growth in the nursery. The combinations high DSD × low LD and low DSD × high LD reached 21 % to 24 % and plants with water and light limitation about 13 %.
Due to the cumulative effect of DSD and LD, the effect of water and light limitation on biomass development was first statistically significant in the third year after transplantation. Increasing total DSD significantly reduced and increasing total LD significantly stimulated the development of shoot biomass (Fig. 2.2 a). Root biomass development, by contrast, was hardly affected by drought and vari-

able light conditions, in the absence of significant correlations (Fig. 2.2 b). Still in consideration of its size dependency (via inclusion of the previous year diameter of the plants as covariate of the regression model), root-shoot-ratio (R/S-ratio) was significantly ($p = 0.028$) increased with increasing total DSD, resulting from the strong reduction of shoot biomass development under drought (Fig. 2.2 c). By contrast, no significant correlation between R/S-ratio and total LD was found. In consistency with the shoot biomass in 2011, nrBAI reflected a significant negative effect of DSD and a significant positive effect of LD on diameter increment throughout the 3-year study period (Fig. 2.3). While effects of water and light limitation were strongest during the first growing season after transplanting 2009, they were mitigated in the subsequent years. Although, due to large variance in the data, testing the inequality of the slopes for DSD and LD across the three years led to non-significant results, analysis showed an evident trend of progressively flattened slopes for DSD and LD from 2009 to 2011 (c.f. Tab. 2.3).

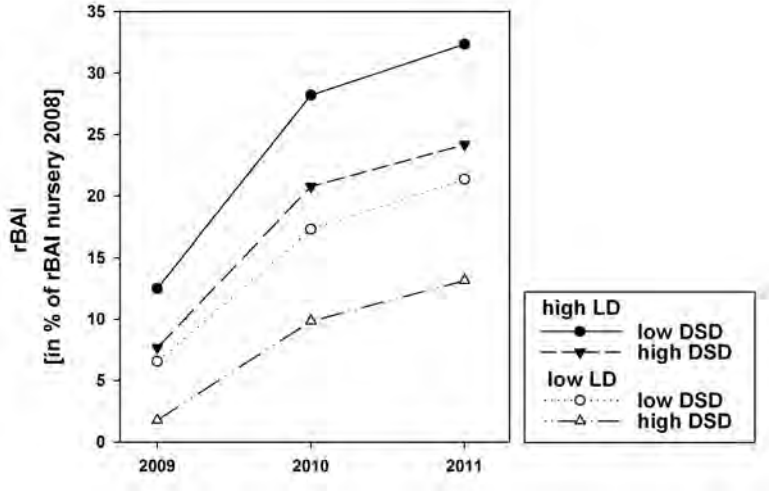

Figure 2.1: rBAI of 2009 through 2011 as percentage of rBAI in 2008 under nursery conditions. Data derived from 3D regressions correlating rBAI with DSD and LD (function and statistics, see Table 2.3); low/high logDSD: 0.1/1.6 MPa*day, low/high logLD: 2.4/3.0 MJ m^{-2}

Specific leaf area and leaf gas exchange

Under open site conditions in the nursery, full sunlight exposure had caused the two-year-old plants to develop low SLA, averaging at 12.2 ($\pm$1.97 SD) $m^2\,kg^{-1}$ (Fig. 2.4). After one growing season (2009) under the shady conditions of the forest stand, SLA continued to increase significantly to 21.8 ($\pm$2.99 SD) $m^2\,kg^{-1}$ in 2010 and 27.7 ($\pm$4.15 SD) $m^2\,kg^{-1}$ in 2011. This response of SLA to shading was also evident along the gradient of total LD. After three years upon transplant, SLA was significantly ($p = 0.04$) increased with decreasing total LD. By contrast, no significant correlation between SLA and total DSD has been found. In parallel to the in-

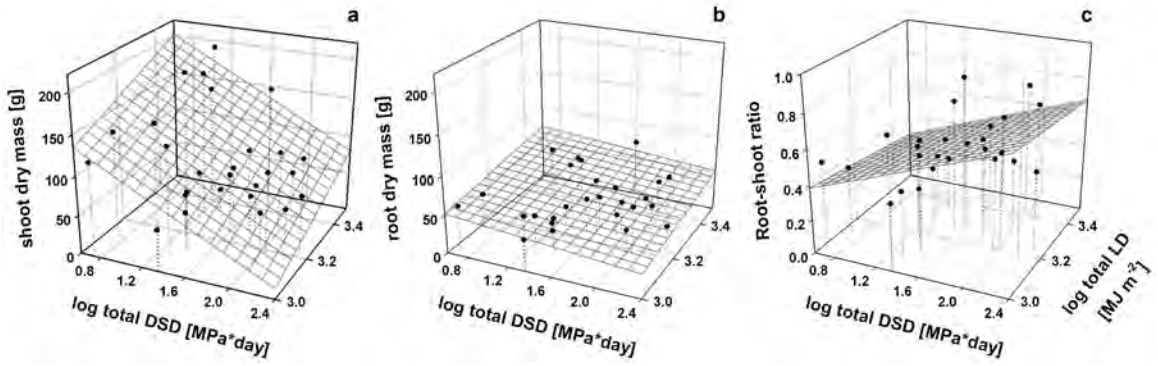

Figure 2.2: Biomass and root-shoot ratio at the end of the three year study period (2011) in relation to total DSD and total LD. Total DSD and total LD as cumulated during growing seasons of 2009, 2010 and 2011. **a)** Shoot dry mass, **b)** root dry mass, **c)** root-shoot ratio. Linear 3-dimensional regressions: function and statistics see Table 2.3.

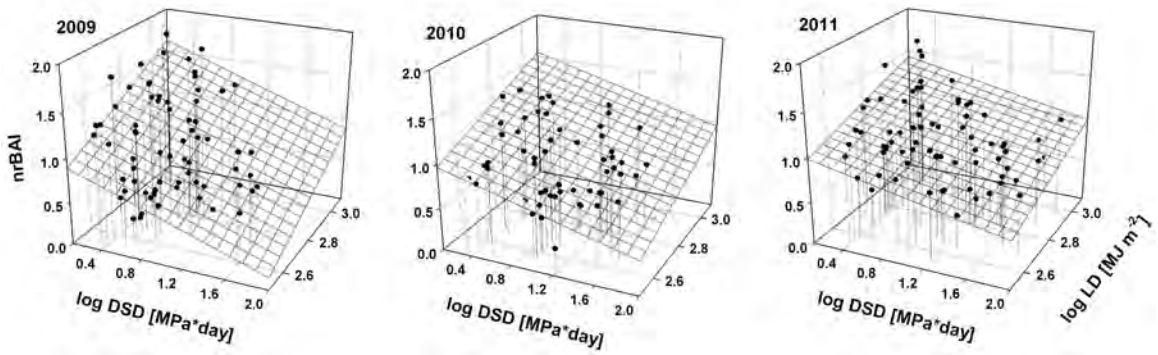

Figure 2.3: Normalized relative basal area increment (nrBAI) in relation to annual DSD and LD from 2009 to 2011 with rBAI, derived from tree ring analysis of plants harvested in 2011. Linear 3-dimensional regressions: function and statistics see Table 2.3.

crease of SLA, both, area based net CO_2 uptake rate of leaves under non-limiting water and light supply (A_{max}) and dark respiration rate (R) were significantly reduced, from average A_{max} of 13.1 ($\pm$1.08 SD) μmol (CO_2) m^{-2} s^{-1} at nursery to 9.7 ($\pm$1.71 SD) μmol (CO_2) m^{-2} s^{-1} in 2010 and 9.8 ($\pm$1.74 SD) μmol (CO_2) m^{-2} s^{-1} in 2011. R declined from 2.1 to 0.5 ($\pm$0.15 SD) μmol (CO_2) m^{-2} s^{-1} in 2010 and 0.4 ($\pm$0.18 SD) μmol (CO_2) m^{-2} s^{-1} in 2011 (Fig. 2.4 a). In contrast to A_{max} on leaf area basis, mass-based A_{max} progressively increased from 0.16 ($\pm$0.03 SD) μmol (CO_2)g^{-1} s^{-1} under nursery conditions to 0.21 ($\pm$0.05 SD) μmol(CO_2)g^{-1} s^{-1} in 2010 and 0.27 ($\pm$0.07 SD) μmol(CO_2)g^{-1} s^{-1} in 2011 (Fig. 2.4 b).
Comparisons revealed that gas exchange parameters correlated better with instantaneous water and light availability than with DSD and LD. The net CO_2 uptake rate followed a hyperbolic function towards light saturation, reaching max-

imum levels of about 10 μmol (CO_2) m^{-2} s^{-1} at non-limiting water availability (Fig. 2.5 a, d). Decreasing soil water potential decreased the CO_2 uptake rate in 2010 and 2011. During the latter year, however, the effect of drought was weaker than in 2010. At light saturation, 10 % reduction in CO_2 uptake rate was caused by soil water potentials of less than -0.19 MPa in 2010, whereas such a reduction was reached in 2011 only at soil water potentials of less than -0.51 MPa. Decreasing soil water potential led to stomatal closure (Fig. 2.5 b, e) and consequently to CO_2 assimilation at higher water-use-efficiency (Fig. 2.5 c, f). In 2011, even at high soil water potentials, stomatal conductance was generally lower than in 2010. Regressions of A_{net} in correlation with g_s showed significantly lower slopes in 2010 ($d\,A_{net}(g_s)/d\,g_s = 0.016$) than in 2011 ($d\,A_{net}(g_s)/d\,g_s = 0.051$) (regression data not shown). As a consequence of similar CO_2 uptake rates at generally lower stomatal conductance, overall higher iWUE prevailed in 2011 than 2010.

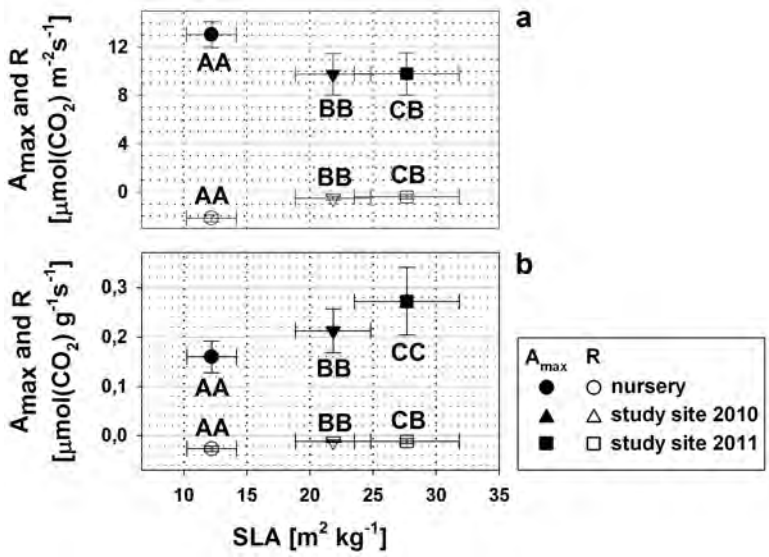

Figure 2.4: Specific leaf area and corresponding leaf CO_2 gas exchange. A_{max}: leaf CO_2 assimilation rate on area **(a)** and mass basis **(b)** under non-limiting light (PPFD $>$ 800 μmol(photons) m^{-2} s^{-1}) and water supply ($\Psi_{soil} >$ -0.02 MPa), R: leaf dark respiration on area **(a)** and mass **(b)** basis. SLA: specific leaf area from harvested plants. Arithmetic means ±standard deviation. Different letters indicate significant differences (one factorial ANOVA) between samples (years) at $p < 0.05$, while first letter refers to SLA and second to A_{max} or R respectively.

Root growth and morphology

Rooting depth was significantly increased with increasing total DSD (Fig. 2.6 a), in the absence of significant correlation with total LD. Contrasting the significant decrease of the mean fine root diameter with increasing total DSD (Fig. 2.6 b), there was no significant correlation between specific root length SRL and total

Table 2.3: Specification of used regression functions and statistical values of the shown graphs; ns = not statistically significant, (*) = statistically significant trend ($p < 0.1$), * = statistically significant ($p < 0.05$), ** = highly statistically significant ($p < 0.01$); - = data not available/shown; pModel = p of overall regression; p_{diff} = p of difference to slope of 2009.

		2009	**2010**	**2011**	**n**
shoot dry mass z = a+bx+cy	adj.R^2			0.21	
	pDSD	-	-	**	30
	pLD			(*)	
root dry mass z = a+bx+cy	adj.R^2			0.003	
	pDSD	-	-	ns	30
	pLD			ns	
R/S-ratio z = a+bx+cy	adj.R^2			0.16	
	pDSD	-	-	*	30
	pLD			ns	
mean fine root diameter z = a+bx+cy	adj.R^2			0.207	
	pDSD	-	-	*	60
	pLD			(*)	
rBAI z = a+bx+cy	adj.R^2	0.18	0.23	0.21	
	pDSD	**	**	**	60
	pLD	**	**	**	
nrBAI z = a+bx+cy	adj.R^2	0.17	0.24	0.22	
	pDSD	**	**	**	
	pLD	**	**	**	
	slope DSD	-0.39	-0.28	-0.23	60
	slope LD	1.24	0.98	0.73	
	p_{diff} DSD	-	ns (0.43)	ns (0.24)	
	p_{diff} LD	-	ns (0.60)	ns (0.30)	
A_{net} z = a+bx+cy^2+d exp(-exp(e-x)/f)	adj.R^2	-	0.85	0.73	2010: 22;
	pModel		**	**	2011: 46
g_s z = a exp(-0.5((ln(x/b)/c)^2+((y-d)/e)^2))	adj.R^2	-	0.24	0.25	2010: 22;
	pModel		**	**	2011: 46
iWUE z = a+by+cy^2+d/(1+((x-e)/f)^2)	adj.R^2	-	0.62	0.46	2010: 22;
	pModel		*	*	2011: 46

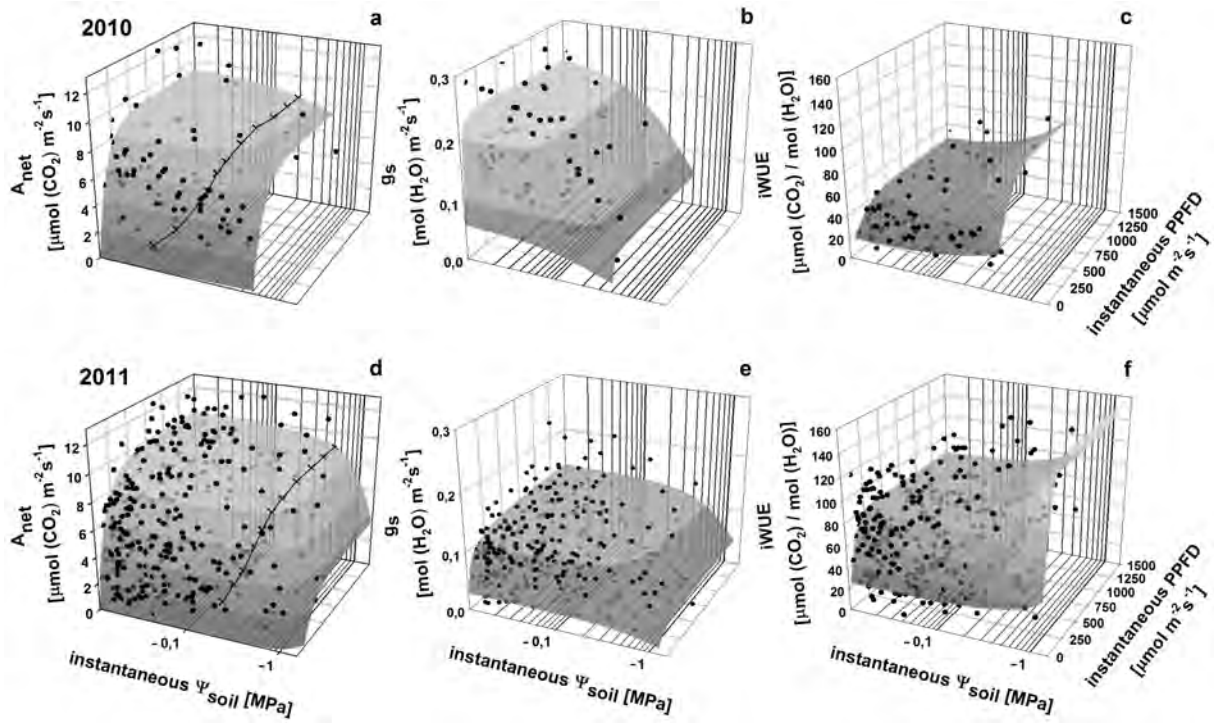

Figure 2.5: Discrete leaf gas exchange measurements from June through end of August in correlation with instantaneous soil water potential and photon flux density. **a), d)** Leaf CO_2 net assimilation rate (A_{net}), black line indicates soil water potential leading to a 10 %-reduction of leaf CO_2 assimilation at different light availability. **b), e)** Stomatal conductance to water vapor (g_s). **c), f)** Intrinsic Water-use-efficiency (iWUE). Non-linear 3-dimensional regression: function and statistics see Table 2.3.

DSD (data not shown). However, correlations showed significant trends towards increased SRL (data not shown) along with a co-occurring decrease of the mean fine root diameter with increasing total LD.

2.5 Discussion

Assessment of DSD and LD and analysis along prevailing gradients

In the present study, small plant size limited the assessment frequency of predawn water potential to determine plant water status, as methods for quantifying plant water potential are either destructive (e.g. Scholander pressure bomb or psychrometry of leaves) or highly invasive (e.g. stem psychrometry). In this context, continuous measurements of volumetric soil water contents and its transformation to soil water potentials via individual calibration resulted in a valuable proxy for plant available soil water under the given heterogeneous soil conditions in the field. Referring to the present highly significant correlation between soil water

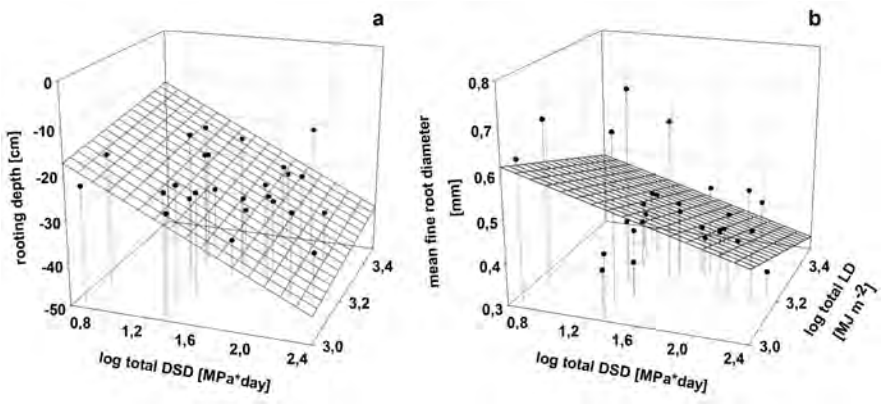

Figure 2.6: Root traits of the plants harvested in October 2011. **(a)** Maximum rooting depth (root diameter > 2 mm), **(b)** mean fine root diameter. Linear 3-dimensional regressions: function and statistics see Table 2.3.

potentials and leaf predawn potentials and the data on rooting depth three years upon transplant, it can be assumed that the data of the FDR probes installed into a depth of 30 cm mineral soil are representative for the plant available soil water in the overall rooting profile. Apart from better comparability within the present experiment, individual calibration and transformation of volumetric soil water content into soil water potential can facilitate the comparability of the present results with other drought experiments (Vicca et al., 2012). Continuous data allowed the calculation of the cumulative drought stress and the analysis of response patterns along the induced gradient of DSD.

Stand restoration situations upon wind throw typically implicates heterogeneous light conditions due to a patchy distribution of the remnants of the old stand. According to Balandier et al. (2007), studies on the effect of varying irradiances on growth of recently transplanted juvenile beech have to extend over at least two years to obtain significant and reliable results. However, especially in the context of global change, it is important to gain a better understanding of plant response patterns during the high risk period directly upon transplant. Despite the large variance in our dataset, the applied regression analysis revealed significant and temporarily consistent effects of water and light availability on plant growth and leaf gas exchange. This highlights the additional information obtained by the present gradient approach. Dose response relationships, as assessed in the present approach, enable to scale for the regarded factor and are advantageous when comparing results with other experiments investigating the plant's response on the given environmental factor (Poorter et al., 2012).

Interrelations of light availability, progressive light acclimation and growth limitation under drought

Confirming hypothesis (i), plant biomass development and rBAI reflected different combinations of light and water availability to have an additive impact on plant growth, with lowest plant growth under co-occurring light and water limitation. Such an outcome prevailed even after three years of acclimation to shade and drought. Light availability below light saturation of photosynthesis confined carbon assimilation and adjustment towards high iWUE, implying an additionally intensified trade-off between carbon gain and water loss under conditions of co-occurring drought. This adjusts to the study on beech seedlings by Robson et al. (2009) who found higher growth rates at higher iWUE in forest gaps than in the understory. Another aspect which may account for the reduction of growth performance under co-occurring light and water limitation is that light limitation can impede osmotic adjustment of young beech, constraining an important drought tolerance mechanism (Aranda et al., 2001a; Robson et al., 2009).
In 2009 and 2010, subsisting high-light acclimation from the nursery and its negative implications on net carbon gain (i.e. increased light compensation point and dark respiration; c.f. Björkman (1981) and Rodríguez-Calcerrada et al. (2010)) under shade, may account for the reduction of plant growth with increasing light limitation to be strongest in the first year upon transplant. This result is supported indirectly by the findings of Welander and Ottosson (1998) on seedlings of *Quercus robur*, where the positive effect of increasing light availability on biomass growth was greater for previously high-light acclimated seedlings than for previously low-light acclimated seedlings. For beech in particular, Eschrich et al. (1989) and Thiebaut et al. (1990) document carry-over effects on leaf morphology to be dependent on previous-year light exposure. Present results on SLA indicate that progressive light acclimation was not confined to the first growing season upon transplantation. This observation is coherent with observations of Roloff (1987), showing a biennial morphogenetic cycle from initiation of bud formation until bud break. Referring to Chinnusamy and Zhu (2009), Nicotra et al. (2010) and Thellier and Lüttge (2013), epigenetic modifications due to acclimation to environmental conditions can stay active for prolonged periods. In the third year upon transplantation SLA resembled to that reported by Čater et al. (2012) from *F. sylvatica* saplings after 13 years of acclimation to shading levels as given in the present study, so that three years are concluded to enable complete acclimation of SLA to prevalent light conditions. Effects of nutrition on SLA can be neglected, as there was no indication of severe nutrient limitation of the plants Poorter et al. (2009).
Our findings on leaf area-based A_{max} and R in correlation with the increase of SLA adjust to the generally reported shift from maximizing carbon gain through maximization of photosynthetic capacity under high light exposure towards max-

imization of light interception efficiency under low light conditions. Contrary to results for a wide range of other plant species (Evans and Poorter, 2001; Poorter et al., 2009) showing mass-based A_{max} to be independent of SLA, in the present study mass-based A_{max} was increased with increasing SLA. Hence, indicating an increasing potential to fix carbon along with proceeding shade acclimation. Similar results for *Fagus sylvatica* were previously reported by Gardiner et al. (2009). In the context of shade acclimation, an increase in mass-based leaf nitrogen (Gardiner et al., 2009) and chlorophyll content (Valladares and Pearcy, 2002) has been reported for young beech, which could be causal for present higher mass-based Amax of shade acclimated leaves.

Under water limitation, maximization of the water-use-efficiency is a crucial response to mitigate the trade-off between transpiration and carbon assimilation. At similar CO_2 assimilation rates in 2010 and 2011, we found prevalently higher iWUE in 2011 than in 2010 to be the consequence of overall lower g_s in 2011. In the context of preceding shade acclimation from 2010 to 2011, this result conflicts with Welander and Ottosson (1997) who found higher transpiration rates for previously low-light acclimated beech seedlings, in comparison with previously high-light acclimated seedlings and with the results of Aranda et al. (2007) on *Quercus suber*, showing a negative correlation between SLA and WUE. However, Valladares and Pearcy (2002) and Abrams and Mostoller (1995) show for *F. sylvatica* and for six North American hardwood tree species that acclimation to low light does not only increase SLA, but also decrease stomata density and length. This observation is in agree with Aranda et al. (2004), reporting a reduced maximum stomatal conductance of juvenile beech under shady conditions in the understory of a pine stand, when compared with the conductance under increased radiation in thinned plots.

That an experienced drought stimulus can significantly reduce stomata index and stomatal conductance of subsequently produced leaves, due to induced shifts in the genetic program that underlies the formation of stomata, showed Hamanishi et al. (2012) for *Populus balsamifera*. In the present case, such shade and drought induced alterations in leaf morphology, in combination with the increase in carbon fixation potential between 2010 and 2011, could account for the observed overall reduction of area-based gs and consequently increased iWUE in 2011.

Biomass partitioning and fine root morphology under light and water limitation

Above versus below-ground biomass partitioning may relate to three drivers: First, the ontogenetic drift in R/S-ratio, promoting biomass increment of the shoot (Poorter et al., 2012). Second, regeneration of the root system upon transplantation (Burdett, 1990; Grossnickle, 2005). And third, root-shoot carbon allocation as a means of acclimation to water and light availability (Löf et al., 2005; Poorter et al., 2012; van Hees, 1997).

Although the three drivers were inter-related in this study, our results show that R/S-ratio was significantly increased under progressive water limitation in the absence of light effects at three years after transplantation. The missing light effect contrasts with results from previous studies on *F. sylvatica* (Schall et al., 2012; Valladares and Pearcy, 2002; van Hees, 1997; Welander and Ottosson, 1998) and the meta-analysis of Poorter et al. (2012) which confirm the concept of a *functional equilibrium*, that implies a relatively enhanced allocation to the shoot under limiting light availability. Remarkably, non-limiting water availability enhanced shoot growth, in the absence of significant effects on roots. This implies that plants under limiting conditions allocated relatively more carbon to the root compartment. Supposedly upon transplantation, for all plants the urgent need to regenerate their root system was mainly determining the carbon partitioning between root and shoot. Rooting depth was increased with increasing DSD, indicating spatial changes of root growth for accessing water resources in vertical direction. In numerous studies, evidence for selective root foraging as response to patchy resource availability was found (Hodge, 2004) and increased rooting depth in particular is regarded to be an important stress-avoidance strategy under water limitation (Reader et al., 1993). Such conclusions are confirmed by the present study, although conflicts remain with the rhizotron-experiment of Meier and Leuschner (2008) who did find rooting depth of beech to be decreased under drought. Fine root morphology responded to water limitation. Roots displaying high SRL, are conducive to efficient and competitive for water and nutrient uptake, i.e. such roots exhibiting high surface area per unit carbon costs (Grams et al., 2002). Nevertheless, root elongation may be impeded and tissue density increased by increasing impermeability of drying soil, biasing SRL differentiation under severe soil drought (Hodge, 2004; Ostonen et al., 2007). In addition, light availability positively correlates with SRL in beech (Čater, 2010; Curt et al., 2005). Our data confirmed such patterns. The formation of thinner fine roots was promoted with increasing DSD and LD, similar to findings by Montagnoli et al. (2012) from *Quercus cerris*. Although we found increasing LD to affect fine root morphology towards an efficient water and nutrient acquisition under drought, we have to reject hypothesis (ii) as light exposure had no significant effect, neither on R/S-ratio nor on rooting depth.

Water and light limitation and intensity of planting shock

Unfavorable conditions at the forest site, characterized by lower mean temperature, stronger competition for resources and a shorter growing season than in the nursery, apparently limited plant growth. For example growth limitation is indicated by lowered rBAI still three years after transplantation. In agreement with Burdett (1990) and Grossnickle (2005), growth reduction due to the planting shock was enhanced under water limitation. Low light availability ad-

ditionally limited growth. Given the optimal growth conditions in the nursery, the plants had to adjust their metabolism and morphology to the conditions at the plantation site. Recovery from planting shock can be described as a *positive cycle of root growth and photosynthesis* (Burdett, 1990). Burdett's conceptual model explains the present findings on rBAI at different combinations of water and light availability: Plants under non-limiting conditions showed relevant growth during the first growing season upon transplantation at high recovery, whereas plants under limiting water and light conditions initially showed almost disrupted growth. Hence, growth analysis confirmed hypothesis (iii) about intensifying planting shock under water and light limitation.

2.6 Conclusions and implications for silvicultural practice

According to Holmgren et al. (2012), for shade-tolerant and drought-sensitive tree species such as *Fagus sylvatica* shade should ameliorate negative effects of water limitation. Our results highlight that, such generalizations have to be used carefully and that previous light acclimation substantially modifies response patterns to water limitation under changed light conditions upon transplantation. Co-occurring water and light limitation induce conflicting acclimation responses that optimize light capture on the one hand and water stress tolerance on the other hand (e.g.: increased/reduced above ground growth (Poorter et al., 2012), lower/higher osmoregulation capacity (Aranda et al., 2001b, 2005)). Present results imply that such conflictive response patterns impede the positive feedback of root regeneration and photosynthesis, hence confining productivity upon transplant. Shade acclimation may lead to increased xylem vulnerability to cavitation, causing a higher drought related risk due to xylem disfunction (Cochard et al., 1999). However in the present study, despite very low soil water potentials in 2011, no drought induced mortality has been observed.
We therefore conclude that a broader assortment of plant material acclimated to different light exposure and more detailed information about the light regime in the designated forest stand could help to ensure adequate light acclimation of nursery plants and hence increasing success of stand establishment under coinciding drought events in the first growing seasons upon transplantation.

The most important implications of the present study for forest conversion with nursery derived juvenile beech are:

- High-light acclimation in the nursery affect leaf morphology and hence leaf gas exchange up to two growing seasons upon transplantation.

- Light limitation upon transplantation to shady stand conditions leads to enhanced growth limitation under drought.
- Both, light and water limitation intensify the planting shock upon transplantation.
- Despite the strong negative effect on plant growth, no drought or shade induced mortality was observed.

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3 Planted beech saplings (*Fagus sylvatica* L.) under repeated soil drought: high resilience upon transplantation

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3.1 Abstract

European beech saplings (*Fagus sylvatica* L.) are considered drought-sensitive which raises concern about the species' fitness under changing climate. However, the assessment of drought stress on plant level is difficult as morphological and chemical traits of aboveground and belowground plant compartments respond differently to drought stress. Here, we quantified and categorized the response of several plant traits to soil drought of transplanted juvenile beech in a typical reforested site. In an open 140-year-old spruce forest, underplanted beech saplings were experimentally subjected to different levels of soil drought during three growing seasons. Spatial gradients of soil water availability resulted from natural pattern of throughfall, exclusion by rain shelters underneath the spruce stand canopy and soil irrigation with a sprinkler system. We used cumulated soil water potential as a quantitative measure for drought (*drought stress dose*, DSD), calculated for three different periods: 'year of harvest', 'year before harvest' and 3-year average. Morphological and chemical plant parameters including δ^{13}C of buds as commonly used for drought indication, were subjected to multivariate regression analysis. δ^{13}C of buds was used as a reference for sensitivity characterization of different parameters to drought.

Rooting depth and Mg concentration in fine roots exhibited highest correlation with DSD, being more reliable for drought indication than δ^{13}C of buds. Statistical analysis identified parameters either indicative of recent (e. g. leaf C/N-ratio) or past drought events (e. g. specific leaf area, total non-structural carbohydrates in fine roots). Several parameters reflected long-term average of soil drought (e. g. fine root C/N-ratio, abundance of mycorrhizal root tips) as opposed to such of minor or absent response after three growing seasons (e. g. root/shoot ratio, many non-structural carbohydrates).

Acclimation of plant traits improves the resilience of beech saplings against drought in the years after transplantation supporting the fitness of juvenile European beech under drier climatic conditions.

3.2 Introduction

European beech (Fagus sylvatica L.) is the dominant tree species in Central Europe's potential natural vegetation (Ellenberg, 1996). Current silvicultural practice aims at converting conifer monocultures to mixed-species forests, with beech increasingly gaining in importance (Tarp et al., 2000). In view of prognosticated climate change with increasing probability of severe summer droughts (IPCC, 2007), the susceptibility of beech to soil drought demands clarification (Gessler et al., 2004), especially regarding juvenile beech which is claimed to be drought-sensitive (Fotelli et al., 2001, 2003; Lendzion and Leuschner, 2008).

Experimental approach has been requested that (1) considers different plant organs as stress indicators (Leuschner et al., 2001; Ryan, 2011), (2) mimics a typical reforestation situation (3) considers repeated and different drought scenarios in consecutive years and (4) employs a physiologically significant and referable measure for drought stress (Vicca et al., 2012).
A variety of morphological drought responses are known that also apply to European beech. Aboveground, decrease of e.g. specific leaf area (SLA) or premature leaf shedding are common (van Hees, 1997; Löf and Welander, 2000; Huang et al., 2008), whereas responses in root morphology to drought are less consistent and only scarcely reported. Fine roots directly face soil water availability and are believed, therefore, to possess indicator functions (Cudlin et al., 2007) so that, e.g., fine root morphology of drought stressed plants is claimed to acclimate towards enhancing water exploitation of soil (Eissenstat and Yanai, 1997). However field observations are not uniform, given high plasticity in plant response as reinforced through genotype, ontogeny, phenology and site scenario (Leuschner et al., 2004; Vanguelova et al., 2005; Ostonen et al., 2007; Meier and Leuschner, 2008; Matyssek et al., 2012). Additionally, the plant's nutritional status is prone to drought stress. Besides changed availability of nutrients in soil solution and ion uptake during drought, ion composition in different plant compartments can actively be altered by metabolic processes, enzyme-driven osmoregulation and electrical charge balancing (Peuke and Rennenberg, 2004, 2011; Maathuis, 2009). Levels of non-structural carbohydrates (NSC) may indicate drought stress as impaired tissue formation can induce NSC accumulation (Körner, 2003). Prolonged drought, however, may consume NSC reserves (McDowell, 2011). Additional information can be mirrored in NSC composition, e.g., through the size of starch reserve pools versus free sugar levels involved in osmoregulation (Kameli and Lösel, 1993; Galvez et al., 2011). Transplantation of beech saplings from nursery to forest site conditions may cause, in addition, enhanced susceptibility to soil drought because of root injury, deficient root/soil connectivity and time needed for acclimation to new site conditions (Burdett, 1990; Coll et al., 2004; Grossnickle, 2005).
Beyond pot experiments (e.g. Löf et al., 2005), knowledge is lacking on beech performance under soil drought, especially during sapling establishment in forest stands. Furthermore, comparability between drought experiments needs to be ensured, as many studies only supply qualitative information on drought stress (Vicca et al., 2012). In preceding studies (Zang et al., 2013; Goisser et al., 2013), cumulative soil water potential over the growing season (drought stress dose, DSD) was closely related to plant parameters of European beech saplings such as radial growth, $\delta^{13}C$ in leaf and soil respiration and rhizosphere respiration. However, DSD does not necessarily reflect the physiologically effective drought intensity as, e.g. below some threshold range, drought response may stay absent

(Granier et al., 2007; Vicca et al., 2012). Also, plant organs or physiological processes may differentially respond to drought (Ryan, 2011), including differing response time (Löf and Welander, 2000; Ammer, 2003; Balandier et al., 2007). Fotelli et al. (2003) explored the relation of different time integrals of soil water potential to $\delta^{13}C$ of different beech tissues, mostly finding higher correlation with 8-week-integrals than shorter time spans. Moreover, buffer capacities of reserve pools can delay stress response Leuschner et al. (2004). Drought reduces the discrimination of $^{13}CO_2$ during photosynthesis, so that $\delta^{13}C$ patterns of plant organs can be useful in characterizing physiologically effective drought stress Fotelli et al. (2003); Meier and Leuschner (2008). As $\delta^{13}C$ comprehends time integrals, its signature in newly grown tissues may mirror the effect of cumulative drought, i.e. of the drought dose; it is thus considered as a rational reference for the characterization of the drought sensitivity of other plant traits. In this study, we aim to analyze the response of individual beech saplings to drought stress for a variety of morphological and chemical plant traits under field conditions in three consecutive years. We assess and categorize the suitability of these plant traits for drought indication in respect of the response characteristics observed for $\delta^{13}C$ in buds.

3.3 Materials and Methods

Study site and experimental set up

The study was performed in a thinned Norway spruce (*Picea abies* (L.) H. Karst) plantation (145 trees per hectare, tree age 140 years) in North-Eastern Bavaria, Fichtelgebirge, Germany (50° 8' N, 11° 52' E, 770 m a. s. l.). Mean annual precipitation accounts for 1160 mm at a mean annual air temperature of 5.3 °C. The soil at the site is classified as a Haplic Podsol, its organic layer corresponds to a moder humus form with a thickness of 7 - 10 cm. For further site description, see Gerstberger et al. (2004) and Hentschel et al. (2007).

Throughfall manipulation and drought quantification

In autumn 2008, two-year-old beech saplings of a local provenance were transplanted to the site at a density of 2,500 plants per hectare. Soil water availability was manipulated by throughfall manipulation during the growing seasons from 2009 to 2011. Nine subplots (400 m^2 each) were subjected to three treatments (n = 3): a) irrigation with deionized water maintaining a soil water potential $>$ -0.02 MPa in the organic layer throughout the year, b) throughfall exclusion during summer months by closing a roof construction with transparent panels and c) non-treated plots representing natural conditions at the site. The manipulations served for adjustment of broad soil moisture gradients.

60 randomly chosen beech saplings from all nine plots were equipped with a FDR soil moisture sensor (10-HS, Decagon Devices, USA) that was vertically installed underneath at 10 - 20 cm depth of the mineral soil. The sensor signal [mV] was logged hourly. Soil water potential > -0.3 MPa was measured 5 to 15 times per plant during each growing season with a tensiometer (T5, UMS GmbH, Germany), and at < -0.3 MPa with a psychrometer (WP4, Decagon Devices Inc., USA). Latter measurements were carried out in a climate chamber (20 °C) with soil samples (5 g) taken from the same soil depth. By correlating soil water potential with the corresponding FDR sensor signal, we deduced individual calibration functions via spline interpolation. With this functions, the time course of soil water potential was modeled during the growing seasons. As a cumulative measure for drought stress, the drought stress dose (DSD) was calculated as follows:

$$DSD = \int \Psi_{soil}(t)\ dt\ \ [\mathrm{MPa\,d}]$$

where $\Psi_{soil}(t)$ is the soil water potential at time instant t; the integration period corresponds to the growing season (May 1 through Oct. 1 of the respective year).

Light conditions

Due to the patchy canopy structure, light conditions were heterogeneous and had to be considered as covariate in the statistical analysis (Jarcuška, 2009). To this end, light exposure of each sapling was assessed by hemispherical photography in summer 2011 (WinSCANOPY DSLR system, Regent Instruments Inc., Canada) in combination with the astronomically defined site-specific time course of global radiation above the canopy (data obtained from a local meteorological tower). The individual light dose (LD) was calculated as follows:

$$LD = \int R_{below\,canopy}(t)\ dt\ \ [\mathrm{MJ\,m^{-2}}]$$

where $R_{below\,canopy}(t)$ is the sum of direct and diffusive radiation reaching the forest floor at instant t. For detailed description see Goisser et al. (2013).

Plant parameters

Leaf samples (n = 10 each) of the 60 beech saplings were taken on August 24, 2011, and immediately frozen at -18 °C. After freeze-drying, the leaf material was milled, homogenized and stored in a desiccator until nutrient and NSC analyses (see below).

The beech saplings were harvested in October 2011, including excavation of the root system. For a subset of 30 saplings, rooting depth was assessed according to the deepest root > 2 mm. The foliage was scanned (600 dpi, 24 bit color depth) im-

mediately after harvest to determine the projected leaf area (SigmaScan 5, Systat Software Inc., USA) and average leaf color ('Leaf area', Hochschule Weihenstephan-Triesdorf, Germany) via graphical analysis. Dry mass of all shoot parts (stem > 2 mm, twigs < 2 mm, leaves and buds) was determined after oven-drying at 60 °C until constant weight. Immediately after harvest, a subsample of fine roots (5 - 10 g) was freeze-dried for NSC and nutrient analyses. Remaining roots and the surrounding soil were stored at 2 °C before, within the following four weeks, the complete root compartment was extracted by washing with tab water. Biomass was separated into living fine (< 2 mm) and coarse roots. A representative subsample of fine roots was scanned at 400 dpi for morphological analysis using the evaluation software WinRHIZO (Regent Instruments Inc., Canada) and visually examined for the proportion of mycorrhizal root tips. After analysis, subsamples were oven-dried at 60 °C together with the other root fractions until constant weight and assessed for dry mass.

Following parameters were calculated for each sapling: Specific leaf area (SLA, leaf area per leaf dry mass, $[m^2\,kg^{-1}]$), leaf area ratio (LAR, leaf area per total sapling biomass, $[m^2\,kg^{-1}]$), leaf mass fraction (LMF, leaf mass per total sapling biomass, $[g\,g^{-1}]$), leaf color (Hue-value, [%]), mean bud weight [mg], twig ramification density $[n\,m^{-2}]$, specific twig length (STL, total twig length per twig weight, $[cm\,g^{-1}]$), root mass fraction (RMF, root biomass per total sapling biomass, $[g\,g^{-1}]$), average fine root diameter [mm], specific fine root length (SRL, fine root length per fine root biomass, $[m\,g^{-1}]$), specific root tip density (SRTD, number of root tips per fine root biomass, $[10^3\,g^{-1}]$), fine root ramification density (=SRTD/SRL, $[m^{-1}]$) and mycorrhizal root tips within whole root tip number [relative abundance, %].

Chemical analyses

For chemical analyses, freeze-dried leaves (harvested end of August 2011) and fine root material (harvested in October 2011) was used. Molar C/N ratio was assessed by combustion analysis (varioEL, elementar Analysensysteme GmbH, Hanau, Germany). Mg, Ca, K and P [μmol g^{-1} dry weight] were analyzed by inductively coupled plasma optical emission spectrometry (Spectro Analytical Instruments, Kleve, Germany) after digestion of the plant material with HNO_3. Water soluble sugars (stachyose, raffinose, sucrose, glucose, fructose) were extracted by hot water at 85 °C and starch was extracted from the remaining pellet after digestion with amylase and amyloglucosidase (Fleischmann et al., 2009). Analysis was conducted using high performance liquid chromatography with a CARBOsep CHO-820 calcium column (Transgenomic, UK). For a subset of 42 saplings, freeze-dried buds were ground and homogenized for $^{13}C/^{12}C$ analysis (delta S, Finnigan MAT, Germany, coupled to an elemental analyzer NA 1108, CE Instruments, Italy).

Statistical analyses

The influence of DSD on morphological and chemical plant parameters was assessed using a multivariate linear regression approach in which LD as well as individual plant biomass were considered as covariates. Plant biomass was implemented as nested effect in order to compensate for possible allometric effects on plant parameters. DSD and LD were log-transformed before analyses. Collinearity of DSD and LD was excluded as the variance inflation factor (VIF) was < 2. 23 data subsets were created with increasing lower limits of DSD (DSD steps) using $\log \mathrm{DSD} = 0.2$ to 1.3 by 0.05 step intervals. The number of replicates decreased from n = 60 (full data set, $\log \mathrm{DSD} = 0.2$ - 1.3) to n = 20 ($\log \mathrm{DSD} > 1.3$).
The influence of DSD on plant parameters was assessed for every data subset by the standardized slope of the regression model (α) and its p-value (p_α) (Fig. 3.1 a and 3.1 c: α and p_α as a function of DSD threshold). Furthermore, as a measure for homoscedasticity and normal distribution of the residuals, the outcome from Breusch-Pagan and Shapiro-Wilk tests, conducted after each regression step, was recorded. Regression analyses were performed with plant parameters from 2011 and three differently defined DSD as explaining variables: (1) DSD during the growing season in which plants were harvested ('DSD_{2011}', 'current-year-drought'), (2) DSD of the preceding growing season ('DSD_{2010}', 'preceding-year-drought') and (3) average DSD of the three growing seasons of experimental throughfall manipulation representing the long-term average of drought ('$DSD_{2009-2011}$', 'average drought') with $DSD_{2009-2011} = 1/3$*(DSD_{2009}+DSD_{2010}+ DSD_{2011}). DSD of the growing season of 2009 was similar to that of 2010 and, hence, not separately tested.

Definition of drought response categories based on ^{13}C signature in buds

We used the ^{13}C signature in buds to define different response categories to soil drought. The study yielded $\delta^{13}C$ of buds to be positively correlated with DSD_{2011} (regression analysis with standardized slope of 0.33 for $\log \mathrm{DSD} = 0.75$; Fig. 3.1 a). The found reduction in the discrimination of ^{13}C is interpreted as resulting from stomatal closure under water limitation (Dawson et al., 2002). As expected, $\delta^{13}C$ in buds is correlated to current-year drought as mainly recent assimilates are invested in bud formation, whereas $\delta^{13}C$ of other plant organs, e.g. leaves or roots, is additionally influenced by the incorporation of reserve C (Hansen and Beck, 1994; Adams and Grierson, 2001). Stomatal closure is among the earliest responses to drought at the leaf level (Chaves et al., 2003). Thus, based on the results of correlation analysis of $\delta^{13}C$ in buds and DSD, we define the drought range of $\log \mathrm{DSD} < 0.75$ as incipient/mild drought and $\log \mathrm{DSD} > 0.75$ as moderate to severe drought. The sensitivity of plant parameters to drought is separated

into weak response (relative to the response of δ^{13}C in buds) with a modulus of standardized slope ($|\alpha|$) < 0.33 and strong response (relative to the response of δ^{13}C in buds) with $|\alpha| > 0.33$ of the multivariate regression analysis (see above). Plant parameters were ascribed to five response categories (Fig. 3.1 b) denoted with 'NR' = no response to DSD (non-significant a for any DSD), 'I' = weak response to incipient drought ($p_\alpha < 0.05$, $|\alpha| < 0.33$, $\log \mathrm{DSD} < 0.75$), 'II' = weak response to high DSD ($p_\alpha < 0.05$, $|\alpha| < 0.33$, $\log \mathrm{DSD} > 0.75$), 'III' = sensitive response to incipient drought ($p_\alpha < 0.05$, $|\alpha| > 0.33$, $\log \mathrm{DSD} < 0.75$) and 'IV' = sensitive response to high DSD ($p_\alpha < 0.05$, $|\alpha| > 0.33$, $\log \mathrm{DSD} > 0.75$). Assignment to the categories I-IV was under the condition of normal distribution of the residuals and homoscedasticity; if one of both was not provided, the regarded parameter was ascribed to category 'NR'.

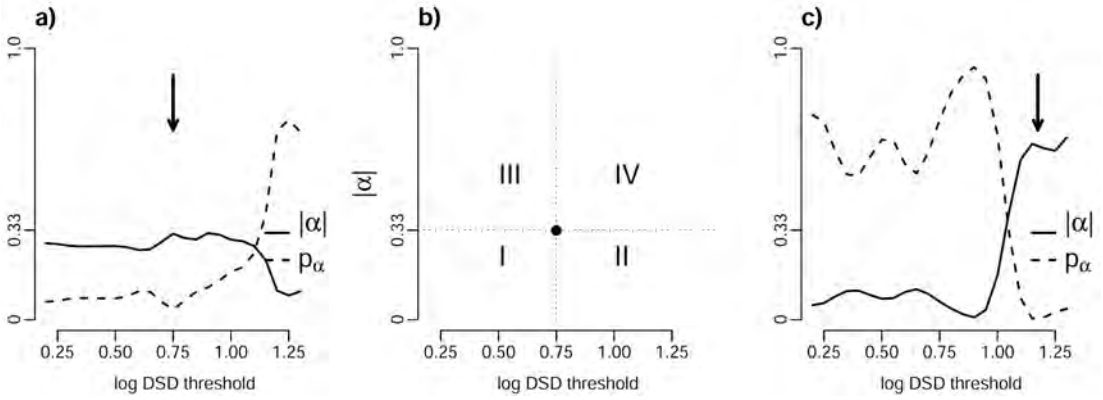

Figure 3.1: a) Visualization of the statistical approach, exemplified for relative abundance of ^{13}C in buds (DSD_{2011}). The diagnostic parameters α and p_α are displayed as a function of DSD threshold. The arrow indicates the DSD threshold yielding minimum significant α. **b)** Visualization of the response categories with 'I' = weak response to incipient drought, 'II' = weak response for high DSD, 'III' = sensitive response to incipient drought and 'IV' = sensitive response for high DSD. The black circle represents the response pattern of δ^{13}C in buds with $|\alpha| = 0.33$ for $\log \mathrm{DSD} = 0.75$. **c)** Visualization of the statistical approach, exemplified for relative abundance of mycorrhizal root tips ($\mathrm{DSD}_{2009-2011}$). The arrow indicates the DSD threshold yielding minimum significant α.

3.4 Results and Discussion

Drought intensity and range of DSD

Minimum soil water potentials in the rooted space of the 60 beech saplings were -0.9 MPa in 2009, -1.2 MPa in 2010 and -1.9 MPa in 2011 (Fig. 3.2). The range of DSD for individual saplings amounted to 1 - 37, 1 - 39 and 2 - 133 MPa d during 2009, 2010 and 2011, respectively. Increase of drought stress is indicated from 2009 through 2011, with broad ranges of water availability each year. Heterogeneity of soil water availability was the result of experimental throughfall manipulation and the distribution of mature spruce trees within the site. Relatively high soil water contents occurred in open areas without protective spruce canopies. Minimum soil water potentials near the wilting point indicate a strong drought level that by far exceeded potential natural drought in this region.

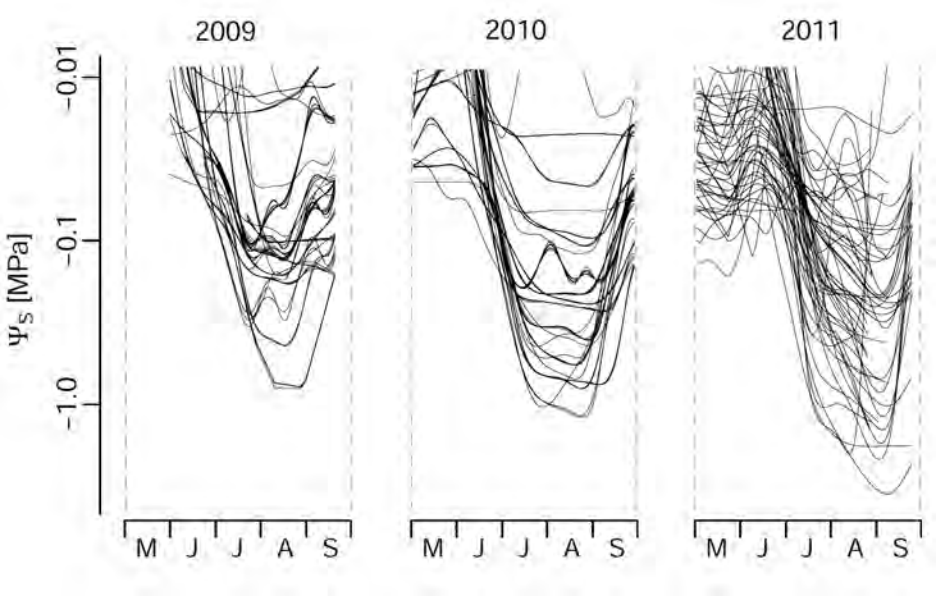

Figure 3.2: Soil water potentials (n = 60) at 10 - 20 cm of mineral soil depth during May through September of the three years of experimental throughfall manipulation.

Morphological parameters

Aboveground morphological parameters

SLA, LAR significantly correlated with DSD_{2010} already upon incipient drought and were ascribed to response category III for DSD_{2010} (Tab. 3.1). Decreasing SLA and LAR reflect increasing sclerophylly which is a common drought response reducing transpirational water loss (Poorter et al., 2009). The delayed response of SLA and LAR, indicated by the correlation with preceding-year drought, strengthens the assumption that leaf size is not only a result of cell turgor during leaf

expansion but is also predetermined by preceding drought events (Löf and Welander, 2000). The concomitant increase of LMF that corresponded to category I (Tab. 3.1) might indicate enhanced C allocation to leaves (Poorter et al., 2012).
Mean bud weight was not correlated with DSD_{2010} and DSD_{2011}; its weak yet significant correlation with $DSD_{2009-2011}$ was positive under severe drought (category II, Tab. 3.1). Sanz-Pérez and Castro-Díez (2010) reported the same trend for Mediterranean oak species, explained by an increased level of abscisic acid during drought (Rinne et al., 1994). As opposed to our findings, preceding-year drought was relevant for bud size in their study.
STL negatively correlated with DSD_{2011} and $DSD_{2009-2011}$ (both response category IV, Tab. 3.1) indicating reduction under prolonged drought. Such morphological response towards compact shoot architecture lowers within-canopy air movement and, hence, transpiration under drought (Rodríguez-Calcerrada et al., 2008). Nevertheless, twig ramification density was not affected by drought (NR).
The Hue-value - quantitatively reflecting visible leaf discoloration - negatively correlated with DSD_{2011} and average drought (category IV and III, respectively, Tab. 3.1). This decline indicated advanced leaf senescence under drought at the time of leaf sampling at the end of August. Premature leaf senescence, together with leaf shedding, is a common phenomenon (Chaves et al., 2003) that occurred in the range of medium to severe drought ($\log DSD$ threshold > 0.75) in our experiment. Furthermore, the correlation of leaf color with $DSD_{2009-2011}$ suggested a long-term drought effect reducing leaf life spans.

Belowground morphological parameters

Mean rooting depth strongly increased with drought from 28 cm for $\log DSD_{2011} < 0.75$ to 47 cm for $\log DSD_{2011} > 1.6$ (data not shown); the correlation with drought was highly significant ($p < 0.01$) and yielded standardized slopes of > 0.64 (data not shown) for current-year, preceding-year as well as average drought (category III in every case, Tab. 3.1). Exploitation of deeper soil layers with generally higher water content was the most prominent drought response of the root compartment which is generally considered as an effective acclimation strategy to increase drought tolerance for many species (Reader et al., 1993; Niinemets, 2010). A considerable sensitivity of rooting depth upon incipient drought is proven by $\log DSD$ thresholds of < 0.75 for DSD_{2011}, DSD_{2010} and $DSD_{2009-2011}$. Our findings contrast with those of Meier and Leuschner (2008) stating repeated summer drought to stay ineffective on rooting depth in *Fagus sylvatica* seedlings. However, the drought range was not quantified rendering a direct comparison impossible. Furthermore, soil penetrability which generally decreases with increasing drought and potentially restricts root growth (Bengough et al., 2006) might be less limiting in granite-derived soil with a sandy to loamy texture as on our study site.

Table 3.1: Means (±SD) of aboveground and belowground morphological plant parameters and categories of drought responses for DSD_{2010}, DSD_{2011} and $DSD_{2009-2011}$. '-' denotes negative correlation with DSD.

	mean±SD	**DSD_{2010}**	**DSD_{2011}**	**$DSD_{2009-2011}$**
aboveground parameters				
SLA [$m^2\,kg^{-1}$]	25.1 (5.1)	-III	NR	NR
LAR [$m^2\,kg^{-1}$]	2.49 (0.43)	-III	NR	NR
LMF [$g\,g^{-1}$]	0.095 (0.021)	+I	NR	NR
mean bud weight [mg]	13.0 (4.0)	NR	NR	+II
twig ram. density [$n\,m^{-1}$]	10.8(2.3)	NR	NR	NR
STL [$cm\,g^{-1}$]	30.8 (7.5)	NR	-IV	-IV
leaf color (Hue) [%]	42 (17)	NR	-IV	-III
belowground parameters				
rooting depth [cm mineral soil]	29.0 (10.0)	+III	+III	+III
RMF [$g\,g^{-1}$]	0.41 (0.09)	NR	NR	NR
r/s-ratio [$g\,g^{-1}$]	0.74 (0.25)	NR	NR	NR
SRL [$m\,g^{-1}$]	15.6 (7.6)	NR	NR	NR
average fine root diameter [mm]	0.46 (0.10)	NR	-I	-I
SRTD [$10^3\,g^{-1}$]	9.6 (7.2)	+III	NR	+III
fine root ram. density [$n\,m^{-1}$]	580 (260)	+III	NR	+IV
mycorrhizal root tips [%]	40 (37)	NR	NR	-IV

The increase of RMF with drought was significant for $DSD_{2009-2011}$ (category III). This points to a shift in C allocation towards the belowground compartment and, in the present study, also results from limited root growth (Goisser et al., 2013). The proportionality between root and shoot changes drastically during the sapling age (Gedroc et al., 1996). Without considering plant biomass as covariate in statistical analysis, the response of RMF to drought would have been overestimated. However, it has been evidenced to be of high sensitivity upon drought.
No significant drought response was observed for SRL in our experiment which is in contrast to other studies that observed increasing SRL with increasing drought, generally interpreted as a strategy to increase water uptake (Metcalfe et al., 2008; Meier and Leuschner, 2008; Montagnoli et al., 2012). Generally, findings on drought responses of SRL under drought have been inconsistent; van Hees (1997) found a decrease in European beech, but an increase in pedunculate oak. We observed a significant decline in average fine root diameter with increasing current-year and average drought (both category I, Tab. 3.1), which may support soil exploitation, even under incipient drought as found in the current study.

SRTD and fine root ramification density increased with DSD_{2010} (both category III) and $DSD_{2009-2011}$ (category III and IV, respectively, Tab. 3.1) while there was no significant correlation to DSD_{2011}. Similar to leaf indices, current-year drought had apparently no effect on SRTD and fine root ramification density although a large portion of fine root tips presumably has been produced during the growing season of 2011. The increase in SRTD and fine root ramification density points to a continuous intensification of soil exploitation when plant-available water is limited (Hishi, 2007). Interestingly, both SRTD and fine root ramification density correlated with DSD_{2010}. To our knowledge, such a carry-over effect of earlier drought events on fine root morphology has not yet been observed.
The decrease of mycorrhizal root tips with increasing average drought (category IV for average drought, Tab. 3.1) is interpreted as a result of C limitation that severely stressed beech saplings have faced. In contrast to our results, Shi et al. (2002) did not observe a reduction of fungal colonization of beech fine roots. Even though, in their study, maximum drought was severe as evidenced by a pre-dawn leaf water potential of -2.4 MPa, we assume that severe drought stress may have been present only during a short time span.

Nutrients

Although low soil water availability may increase nutrient concentration in soil solution and herewith nutrient uptake by plants, nutrient concentrations in fine roots and leaves generally decreased with DSD in our study (Tab. 3.2), perhaps explainable by decreased nutrient mobility under drought (Misra and Tyler, 1999; Wallin et al., 2002). Mg concentration in fine roots significantly decreased with DSD (category III in every case, Tab. 3.2). In contrast, Mg concentration in leaves only correlated with preceding-year drought and only at high DSD (category IV). Apparently, Mg concentration in fine roots was mainly governed by Mg availability in the soil solution, whereas Mg levels stayed buffered in the photosynthetic apparatus (Peuke and Rennenberg, 2004, 2011).
In contrast, relative response of Ca concentration to drought was similar in leaves and fine roots: Negative correlation prevailed at any DSD, although mean Ca concentrations were three time higher in leaves than in fine roots. As in the case of Mg, Ca uptake may have been aggravated by decreasing mobility under drought (Peuke and Rennenberg, 2011).
K concentrations in fine roots appeared to be affected in similar ways, significantly decreasing only with increasing average and preceding-year drought (response category IV and III, respectively, Tab. 3.2). However, it remains open why correlations did not reflect acute drought impact. No drought effects were observed for K concentration in leaves.

Table 3.2: Means (±SD) of nutrient levels in leaves and fine roots and categories of drought responses for DSD_{2010}, DSD_{2011} and $DSD_{2009-2011}$. '-' denotes negative correlation with DSD.

	mean±SD	**DSD_{2010}**	**DSD_{2011}**	**$DSD_{2009-2011}$**
nutrients in leaves				
Mg [μmol g^{-1} dw]	33.7 (17.3)	-IV	NR	NR
Ca [μmol g^{-1} dw]	224 (51)	-III	-II	-IV
K [μmol g^{-1} dw]	183 (49)	-I	NR	NR
P [μmol g^{-1} dw]	48.6 (9.7)	-I	NR	NR
molar C:N-ratio	22.8 (2.5)	NR	+III	NR
nutrients in fine roots				
Mg [μmol g^{-1} dw]	30.9 (9.4)	-III	-III	-III
Ca [μmol g^{-1} dw]	74.9 (27.1)	-III	-I	-I
K [μmol g^{-1} dw]	103 (24.8)	-III	NR	-IV
P [μmol g^{-1} dw]	40.5 (11.0)	NR	NR	NR
molar C:N-ratio	44.8 (6.5)	NR	NR	+IV

Molar C/N-ratio in leaves positively correlated with DSD_{2011} (category III, Tab. 3.2), and in fine roots with $DSD_{2009-2011}$. Increasing molar C/N-ratios both in leaves and fine roots may result from impaired soil N uptake during drought (Nilsson and Wiklund, 1994). Different response patterns can be attributed to life spans of plant organs; fine roots can live to up to several years (Strand et al., 2008) and therefore represent the long-term average ($DSD_{2009-2011}$) whereas leaves rather reflect annual variation in nutrition (DSD_{2011}). Accordingly, C/N-ratios of roots only responded to severe drought, whereas leaf C/N-ratios reflected incipient drought.

P concentration in leaves significantly decreased under drought (response category I, Tab. 3.2) although to a lesser extent than did other nutrients. Conversely, Peuke and Rennenberg (2004) found P as the element most affected by drought due to reduced mobility. On our study site, high P supply, however, prevailed because of granite-derived apatites (Wunderlich et al., 2012). Remarkably, current-year drought failed to explain P concentrations, whereas preceding-year drought apparently was crucial. Fine roots, in contrast, did not exhibit changes in P concentration. In analogy to C/N-ratio, this is explained by higher longevity as compared to leaves. Additionally, optimum nutrient supply of the saplings during the nursery period may have had a persisting effect on the nutritional status.

Non-structural carbohydrates

Sucrose concentration in leaves increased with drought stress (category IV, Tab. 3.3), perhaps indicating osmoregulation (Kameli and Lösel, 1993). However, the correlation was only significant with average drought. Assuming that osmoregulation is an acute drought response, we conclude that sucrose concentration was influenced by other factors weakening the correlation to current-year drought. A strong depletion of starch was observed in leaves under severe drought (category IV for DSD_{2010} and DSD_{2011}, Tab. 3.3). This observation supports McDowell (2011) assuming that starch reserves, after an accumulation phase in early stages of drought and/or mild drought, can get depleted in later stages of drought and/or under severe drought. Apparently, starch depletion resulted from enhanced respiratory C demand as total NSC reserves continuously decreased in fine roots in relation to preceding-year drought (category III, Tab. 3.3). The correlation was absent for current-year drought pointing to a certain inertia of the NSC pools. The oligosaccharides stachyose (category IV) and raffinose (category III and IV, respectively) decreased in fine roots with average and current-year drought, respectively. Share on total NSC was minor although such carbohydrates may be involved in osmoregulation.

Table 3.3: Means (±SD) of NSC in leaves and fine roots and categories of drought responses for DSD_{2010}, DSD_{2011} and $DSD_{2009-2011}$. '-' denotes negative correlation with DSD.

	mean±SD	**DSD_{2010}**	**DSD_{2011}**	**$DSD_{2009-2011}$**
NSC in leaves				
stachyose [mg g^{-1} dw]	7.0 (4.3)	NR	NR	NR
raffinose [mg g^{-1} dw]	7.9 (4.1)	NR	NR	NR
sucrose [mg g^{-1} dw]	36.5 (18.6)	NR	NR	+IV
glucose [mg g^{-1} dw]	13.4 (11.4)	NR	NR	NR
fructose [mg g^{-1} dw]	13.7 (9.2)	NR	NR	NR
starch [mg g^{-1} dw]	15.5 (13.5)	-IV	-IV	NR
total NSC [mg g^{-1} dw]	94.3 (27.9)	NR	NR	NR
% starch of total NSC	15.7 (10.5)	-IV	-IV	-III
NSC in fine roots				
stachyose [mg g^{-1} dw]	8.6 (2.3)	NR	-IV	-IV
raffinose [mg g^{-1} dw]	6.2 (2.3)	NR	-IV	-III
sucrose [mg g^{-1} dw]	13.4 (8.2)	NR	NR	NR
glucose [mg g^{-1} dw]	8.3 (4.2)	NR	NR	NR
fructose [mg g^{-1} dw]	8.7 (4.7)	NR	NR	NR
starch [mg g^{-1} dw]	22.1 (20.2)	NR	NR	NR
total NSC [mg g^{-1} dw]	67.2 (25.2)	-III	NR	NR
% starch of total NSC	27.7 (19.3)	-I	NR	NR

3.5 Conclusions

Spatial gradients of soil water availability in field studies have great potential to identify the natural sensitivity and variability of plant traits to drought stress. Morphological and chemical plant traits respond to drought stress on different time scales and are strongly influenced by confounding factors and site characteristics. Hence, multivariate analysis is a powerful tool to identify the most sensitive plant parameters to drought stress despite great variability caused by interacting factors. DSD allows the assessment of drought effects along spatial and temporal gradients instead of sole treatment comparisons and the identification of plant traits that can serve as drought indicators. Especially rooting depth and Mg concentration in roots exhibited a sensitive response to drought which by far exceeded the response of $\delta^{13}C$ in buds. Owing to such acclimation processes, planted beech saplings, also in the first years after planting, have a pronounced drought resilience which further increases during establishment if saplings experience repeated drought stress in consecutive years.

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4 Effects of drought stress on photosynthesis, rhizosphere respiration, and fine root characteristics of beech saplings: A rhizotron field study

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4.1 Abstract

Soil drought influences the carbon turnover as well as the fine root system of tree saplings. Particularly during the period of establishment, the susceptibility to drought stress of saplings is increased because of incompletely developed root systems and reduced access to soil water. Here, we subjected beech saplings (*Fagus sylvatica* L.) to different levels of drought stress.
Beech saplings were planted in rhizotrons, which were installed in the soil of a Norway spruce forest before bud burst. Soil moisture was manipulated in the following year during May to September. We measured photosynthetic net CO_2 uptake, volume production of fine roots and rhizosphere respiration during the growing season. Biometric parameters of the fine root system, biomass and non-structural carbohydrates were analyzed upon harvest in October.
Photosynthesis and rhizosphere respiration decreased with increasing drought stress dose (cumulated soil water potential) and cumulative rhizosphere respiration was significantly negatively correlated with drought stress dose. Fine root length and volume production were highest at moderate soil drought, but decreased at severe soil drought. The proportion of fine roots < 0.2 mm and the root/shoot-ratio increased whereas the live/dead-ratio of fine roots decreased with increasing drought stress dose.
We conclude that the belowground C allocation as well as the relative water uptake efficiency of beech saplings is increased under drought.

4.2 Introduction

The response of tree saplings to extended drought periods is of relevance for future forest management as the intensity and frequency of summer droughts is expected to increase during the next decades (IPCC, 2007). Drought stress affects the carbon (C) budget and growth of trees, interactions with other environmental factors such as light intensity, air humidity and temperature may influence the drought effect on the C budget (Irvine et al., 2005; Meir et al., 2008; Rühr et al., 2009; van der Molen et al., 2011).
The effects of soil drought on photosynthesis have been intensively studied. Due to stomatal closure and reduced CO_2 assimilation, drought reduces the amount of available C within the plant (Gollan et al., 1986). Also the allocation of assimilated C to different plant organs is affected, e.g. being retarded under drought (Rühr et al., 2009). Plant belowground responses to drought have been studied less explicitly, given the complexity of the root-soil system. A thorough assessment of drought impacts on the plant-soil system requires a holistic view on the involved response mechanisms (Leuschner et al., 2001; Gaul et al., 2008).
Many plants have the ability to acclimate function and morphology of their root system to water deficiency in the soil (e.g. Joslin et al., 2000; Ostonen et al.,

2007; Metcalfe et al., 2008). Promoted fine root production under drought may foster water uptake by increasing root surface area and by exploitation of moister soil regions (Santantonio and Hermann, 1985; Gaul et al., 2008). Drought-induced formation of thin and widely forked fine roots (diameter < 2 mm) therefore reflects an improved water uptake efficiency. However, limited availability of carbohydrates and nutrients or insufficient penetrability of dry soil can restrict root production (Joslin et al., 2000; Bengough et al., 2006; Metcalfe et al., 2008). Moreover, enhanced fine root mortality is a common phenomenon under severe drought (Janssens et al., 2002; Meier and Leuschner, 2008a,b). Crucial is the balance between fine root production and mortality, being susceptible to drought in either way, so that findings may become contradictory regarding fine root turnover under water limitation (Meier and Leuschner, 2008a; Joslin et al., 2000).

Maintenance and growth of roots represent an important C sink of trees and result in respiratory losses in the form of CO_2 (Eissenstat and Van Rees, 1994; Hanson et al., 2000; Janssens et al., 2002). The measurement of root respiration is difficult due to the fact that most fine roots are associated with mycorrhizal fungi and that roots release exudates, mucilage and other organic compounds into the rhizosphere. As a consequence of this methodological difficulty, respiration by roots and heterotrophic organisms which directly depend on the C supply by live roots is often summarized as rhizosphere respiration (e.g. Kuzyakov, 2002; Borken et al., 2006). Because of the dependency on root C transfer, rhizosphere respiration is prone to drought stress (Irvine et al., 2005; Högberg and Read, 2006; Borken et al., 2006).

An important component of the C budget are non-structural carbohydrates (NSC). Drought can lead to an accumulation of NSC when impaired nutrient uptake limits the formation of plant tissues (Körner, 2003). Thus, the amount of NSC may reflect the drought status of trees, although such compounds are perpetually consumed by enhanced fine root production (Gaul et al., 2008), respiratory metabolism and osmotic adjustment, eventually leading to a decline in NSC concentrations in later stages of drought (McDowell, 2011).

The term *drought stress* is not well defined in the literature. Volumetric soil water content, water filled pore space or a qualitative comparison of different measures of soil drought may provide orientation. Such definitions, however, do not characterize plant-available water and restrict the comparability between studies. Here, we suggest the cumulated soil water potential as a conferrable and tree-relevant measure of drought stress, accounting for the time dimension of stress and, hence, a dose-related responsiveness.

European beech (*Fagus sylvatica* L.) is a dominant tree species in Central Europe and will play a crucial role in future silviculture, even though it is known to be drought-sensitive, especially during early stages of establishment (Bolte and

Roloff, 1993; Bréda et al., 2006). As a species with a pronounced phenotypic plasticity (Meier and Leuschner, 2008b), its response to soil drought has been extensively examined (Mainiero and Kazda, 2006; Nahm et al., 2007; Fotelli et al., 2009; Schall et al., 2012). However, the plasticity of planted beech saplings in terms of the C budget together with morphologic traits is barely known under drought conditions.

We conducted a rhizotron experiment with integrated analyses of photosynthesis, shoot respiration, fine root production and rhizosphere respiration of beech saplings at differing soil water availability. Additionally, we assessed biometric parameters and NSC contents of fine roots. We hypothesized that drought stress decreases rhizosphere respiration, increases fine root production and leads to an accumulation of NSC. Furthermore, we hypothesized that beech saplings adjust fine root morphology to drought towards enhanced effectiveness of water soil exploitation.

4.3 Materials and Methods

Experimental setup

The experiment was conducted on a cleared area (10 m^2) within a thinned out mature Norway spruce stand (140 trees ha^{-1}) in the Fichtelgebirge, North-East Bavaria, Germany (50° 8' N, 11° 52' E, 775 m a. s. l.).
Rhizotrons (size: 30 x 45 x 6 cm; total root observation area = 0.27 m^2 per rhizotron) were constructed to observe the growth of fine roots and to measure CO_2 fluxes from the soil compartment and the shoot of beech saplings (Fig. 4.1). Side walls of the rhizotrons were made of transparent polyvinylchloride (PVC). In spring 2009, the rhizotrons were filled with homogenized and sieved (2 mm) soil from the Bw horizon of the study site (haplic Podsol, sandy loam, pH (H_2O) = 4.6, effective cation exchange capacity: 48 $mmol_c\,kg^{-1}$, base saturation: 12 %, C content 1.27 % (Hentschel et al., 2007). Bulk density was adjusted to 1.1 $g\,cm^{-3}$ by compaction, yielding a soil volume of 7.2 l in each rhizotron. One two-year-old beech sapling (*Fagus sylvatica* L.) was planted into each rhizotron (n = 24). The bare-rooted saplings of North-East-Bavarian provenance were obtained from a local nursery. We installed nine additional control rhizotrons without beech saplings for assessment of the CO_2 flux from decomposition of soil organic matter. Each rhizotron was equipped with a FDR soil moisture sensor (ECH_2O 20, Decagon Devices, USA) that was vertically installed to integrate volumetric water contents (VWC) from 10 to 30 cm soil depth. The soil surface was covered with a sandy quartz layer of 4 cm thickness to minimize water losses by evaporation. The rhiztotrons were placed into slots which were embedded in the forest soil to main-

tain a natural temperature gradient. Potential small-scaled variations in light and temperature were compensated by random rearrangement of the rhizotrons every two weeks. Throughout the rest of the year 2009, the rhizotron soil was held at a soil water potential > -0.02 MPa by adding natural rain water. Drainage of the soil was enabled by small holes in the bottom of the rhizotrons. Rhizotrons were covered with wood chips to prevent freezing of the soil during the winter.
In 2010, six weeks after budburst (end of June), a translucent roof (height 1.5 m) was built over the rhizotrons to exclude natural throughfall and to manipulate soil water contents. VWC was logged hourly during the period of throughfall exclusion.

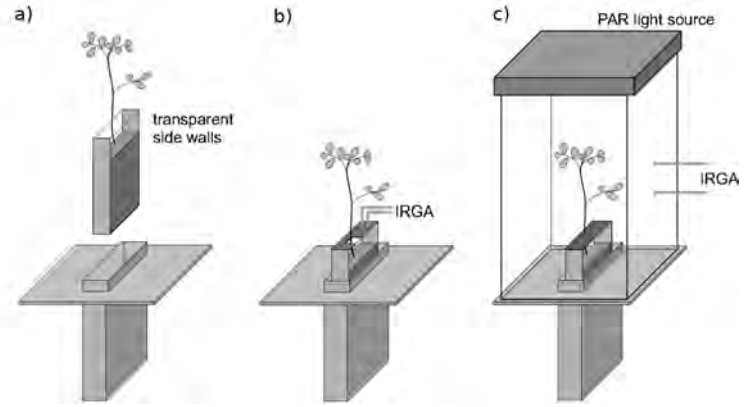

Figure 4.1: Schematic illustration of the rhizotron setup. **a)** general design of rhizotron and slot, **b)** sealed rhizotron during measurement of rhizosphere respiration, **c)** chamber setup for measurement of photosynthesis and aboveground respiration, chamber wall is non-transparent.

Adjustment of soil water potential and quantification of drought stress

Three treatments of soil water availability (n = 8) were established: (A) no water limitation, (B) moderate, and (C) severe water limitation corresponding to mean target soil water potentials of -0.03 MPa, -0.4 MPa and -1.0 MPa, respectively. As shown by preliminary experiments with the same beech provenance, -0.4 MPa represents a level of beginning drought symptoms whereas -1.0 MPa already caused irreversible drought damages at beech saplings. For technical reasons, we chose -0.03 MPa for treatment A rather than field capacity. The rhizotrons were assigned randomly to the treatments. Before the start of individual soil water manipulation, there were no significant differences in shoot diameter and height, abundance of visible roots on the rhizotron side walls, photosynthesis rate and soil respiration between the treatments.
Every one to two days, soil water potential was measured in the rhizotron soil at dawn. A tensiometer was used for soil water potentials > -0.3 MPa (T5 tensiome-

ter, UMS, Germany); measurements were carried out in-situ in 20 cm soil depth. Soil water potentials < -0.3 MPa were assessed using a dewpoint potentiometer (WP4, Decagon Devices, USA). For this purpose, soil samples were taken from 20 cm soil depth of each rhizotron and measured in a climate chamber at 20 °C. Spline fittings of non-linear relationships between soil water potentials and corresponding signals of the FDR sensors [mV] were used to estimate hourly soil water potentials of each rhizotron. The drought stress dose for individual beech saplings was defined as the cumulated soil water potential during the growing season:

$$DSD = -\int \Psi\,(t)\; dt$$

where DSD is the drought stress dose [MPa d] and $\Psi\,(t)$ is the individual time course of soil water potential from budburst to harvest [MPa].
When target soil water potentials were reached after throughfall exclusion, further water losses were compensated by adding deionized water to each rhizotron. The irrigation water was gradually injected through the quartz layer into the soil with a syringe in order to assure a homogeneous distribution of soil moisture. Depending on the transpiration of the beech saplings, the irrigation was conducted by one to three-day intervals at around sunset. The same target soil water potentials were achieved in the rhizotrons without beech saplings by ventilating the soil via a tube connected to the deep soil horizons.

Stomatal conductance

As an indicator of drought stress, stomatal conductance of single leaves (n = 2 per plant) was measured 14, 26 and 64 days after the beginning of the drought treatment at ambient temperature around noon (LiCOR 6400, Licor, USA). The photon flux density was adjusted to 280 μmol m^{-2} s^{-1}.

CO_2 flux measurements

Soil CO_2 efflux was measured on twelve dates from May to October 2010 using the dynamic closed chamber technique. The soil compartment of the rhizotrons was sealed by a lid and an elastic sealant (Terostat, Henkel, Germany) fitted around the beech stem (Fig. 4.1b). CO_2 concentration in the rhizotron headspace (volume 0.95 l) was measured every 10 s over 4 min with an infrared gas analyser (LiCOR 820, Licor, USA). Soil CO_2 efflux was calculated from the slope of the linear regression between CO_2 concentration and incubation time. Rhizosphere respiration arose from the difference in soil CO_2 efflux between planted rhizotrons and rhizotrons without saplings (control).
Net CO_2 uptake rate by photosynthesis was measured with a chamber (volume = 35 l) immediately after the measurement of soil CO_2 efflux. To overcome different light intensities within a day and during the season, we used a light source that was placed on the chamber top plate (area 900 cm^2) and provided photosyn-

thetically active radiation with a constant photon flux density of 250 μmol m^{-2} s^{-1} (Fig. 4.1c). The chamber side walls were covered with aluminum foil to exclude daylight and to prevent the chamber air from heating up by radiation. Net CO_2 uptake was recorded after an equilibration period for light acclimation of >3 min until a linear decrease of CO_2 concentration was observed. During the measurement time of 4 min, the temperature increase of the chamber air was <1.5 °C. Three ventilators inside the chamber ensured sufficient mixing of air during the CO_2 measurement.
Shoot respiration (dark respiration) was assessed on two dates (July 10, August 1) using an opaque chamber in the absence of light. Measurement of CO_2 concentration and flux calculation were carried out in analogy to that of soil respiration.

Root observation

From mid-May until the end of September 2010, both transparent side walls of each rhizotron were photographed on eight dates. Visible roots were analyzed by means of fine root length and diameter using a specific software (WinRHIZO TRON, Regent Inc., Canada). Neither dead nor mycorrhizal roots were identified. We calculated the fine root volume production between two sessions (session i and session i-1) with

$$p = v_{session\,i} - v_{session\,i-1}$$

where p is the fine root volume production and v is the total fine root volume determined at the respective session.

Root and shoot properties after harvest

In October 2010, the complete root system of the beech saplings was extracted by washing with tap water. Fine roots (diameter <2 mm) were separated from coarse roots. Live and dead fine roots were distinguished by means of root color and root tip turgescence. Morphological properties of all live fine roots were determined by scanning (400 dpi resolution) and a digital image evaluation software (WinRHIZO, Regent Inc., Canada). Specific root length (m g^{-1}), relative fine root length distribution by fine root diameter (relative diameter class length, Zobel et al. (2007)) and specific root tip density [g^{-1}] were calculated based on results of the evaluation software and dry mass of fine roots. The latter was determined by freeze-drying immediately after morphological analyses. The fresh leaves were scanned (600 dpi) immediately after harvest to determine the total leaf area (SigmaScan 5, Systat Software Inc., USA). The leaves and all other plant material was oven-dried at 40 °C until constant weight. Root shoot biomass ratio was calculated from the dry mass of all roots and the complete shoot including the foliage. All parameters expressed per unit plant biomass are also based on dry mass.

NSC analysis of freeze-dried fine roots was conducted according to Fleischmann et al. (2009). Water soluble sugars were separated by hot water extraction at 85 °C. Starch was extracted after enzymatic digestion of the remaining pellet with amylase and amyloglucosidase. Analyses were performed with high performance liquid chromatography using a CARBOsep CHO-820 calcium column (Transgenomic, UK).

Statistical analysis

Differences between the treatments were analysed using Tukey's HSD test after analysis of variance ($n = 8$); normality was assumed when data passed the Shapiro-Wilk-test ($p > 0.1$). In case of non-normally distributed data, a Kruskal-Wallis-test was followed by the non-parametric Wilcoxon multiple comparisons test. Additionally, the influence of the individual drought stress dose on plant parameters irrespective of the treatment collective was assessed by linear regression and characterized by the coefficient of determination (r^2) and the p-value of the slope, as well as by Spearman's correlation coefficient. All statistical analyses were performed using R 2.13.0 (R Development Core Team, 2011).

4.4 Results

Time course of soil water potential and stomatal conductance

After the beginning of soil water manipulation in June 2010, soil water potential decreased in all rhizotrons (Fig. 4.2). Mean target soil water potential for treatment A (-0.03 MPa) was reached after two weeks. Thereafter, individual irrigation started. The transient increase in soil water potential at the beginning of August affected all treatments and was due to extreme precipitation from the end of July to the beginning of August. Despite the roof, the rhizotrons were significantly rewetted by lateral rain input, fog and dew deposition. Air temperature and radiation were considerably low so that transpiration did not counterbalance this unintended water input during this period. The drought level from end of July was therefore not re-attained until the end of August. Among the rhizotrons of treatment C, however, minimum individual water potentials of $<$ -1.5 MPa were achieved during a warm period in September.

In July, mean stomatal conductance was consistently enhanced at high soil water availability (Fig. 4.2). Stomatal conductance in treatment C was significantly smaller at the first measurement date and exhibited a minimum of < 0.04 mol H_2O m^{-2} s^{-1}. Reduction of stomatal conductance also occurred in treatment A between the first and second measurement date, but rates were > 0.15 mol H_2O m^{-2} s^{-1}. No significant differences among the treatments were detected in the end of August.

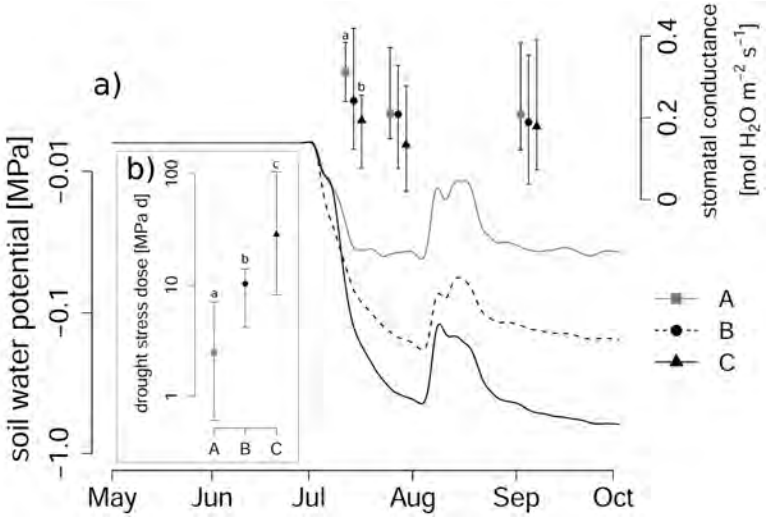

Figure 4.2: a) Time course of mean soil water potential during the growing season of 2010 and stomatal conductance for the three treatments with A = no water limitation, B = moderate drought stress and C = severe drought stress. **b)** Mean drought stress doses (cumulated water potential) for the three treatments calculated for the growing season 2010 from budburst to destructive harvest. Whiskers represent minimum and maximum values; different letters indicate significant differences between the treatments at $p < 0.05$.

Net photosynthesis, aboveground respiration and rhizosphere respiration

In early summer, mean net photosynthesis rate increased along foliage development in all treatments (Fig. 4.3). A 28 % reduction of mean net photosynthesis in treatment C compared to treatment A was observed at the end of July when mean soil water potential in this treatment was close to a local minimum. After wetting in August, photosynthesis recovered in the absence of treatment differences. Small net photosynthesis rates occurred in all treatments as result of leaf senescence in September.

Shoot respiration was not different among the treatments and accounted on average for 11±3.4 % of the net photosynthesis rate (averaged over both measurement dates and all treatments, not shown).

Rhizosphere respiration (net soil CO_2 efflux per rhizotron) followed a typical seasonal pattern and peaked during the first two weeks of July in all treatment (Fig. 4.4). Thereafter, rhizosphere respiration decreased in all treatments, but it was always smaller in the drought treatments. Cumulative rhizosphere respiration (calculated from budburst to harvest) relative to individual root biomass at the end of the growing season negatively correlated with the individual drought stress dose ($p = 0.016$, $r^2 = 0.26$, Fig. 4.5).

Mean CO_2 efflux from control rhizotrons ranged between 0.8 and 5 mg CO_2-C h^{-1} throughout the season. Maximum difference between the treatments was achieved mid of July with 4.9 ± 1.6 and 2.9 ± 1.3 mg CO_2-C h^{-1} for treatment A and C, respectively (difference not significant, data not shown).

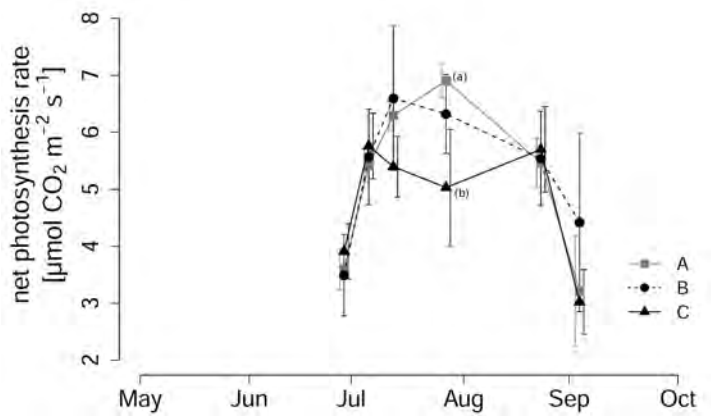

Figure 4.3: Net photosynthesis rate during the growing season of 2010 for the three treatments (mean ± SE, n = 8). Different letters in parentheses indicate differences between the treatments at $p < 0.1$.

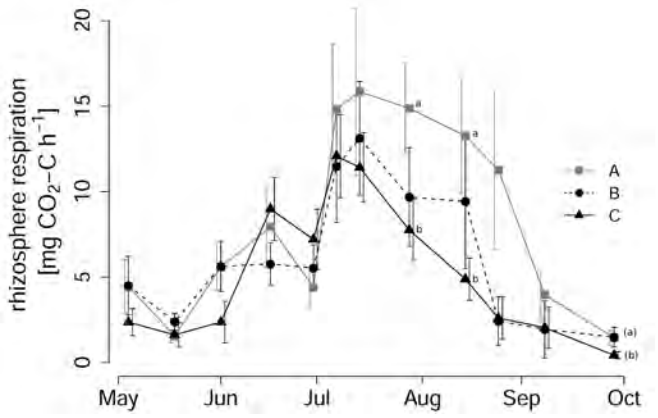

Figure 4.4: Time course of rhizosphere respiration per rhizotron during the growing season of 2010 for the three treatments (mean ± SE, n = 8). Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$.

Fine root production

In all treatments, fine root volume production was variable and increased from May through July (Fig. 4.6a). A subsequent decrease from August until stagnation in September occurred in all rhizotrons. As opposed to treatments A and C, fine root production in treatment B peaked during mid-July. Although not significant, this treatment reached the highest cumulative mean fine root production (calculated from budburst to harvest, Fig. 4.6b).

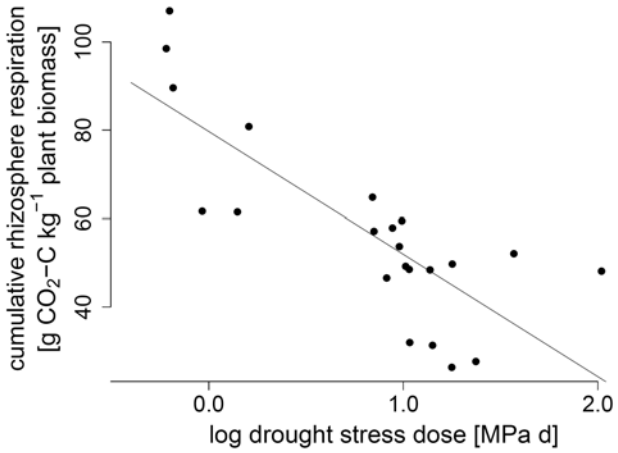

Figure 4.5: Cumulative rhizosphere respiration relative to individual root biomass correlated to the individual drought stress dose (cumulated water potential) ($p = 0.016$, $r^2 = 0.26$, $n = 22$)

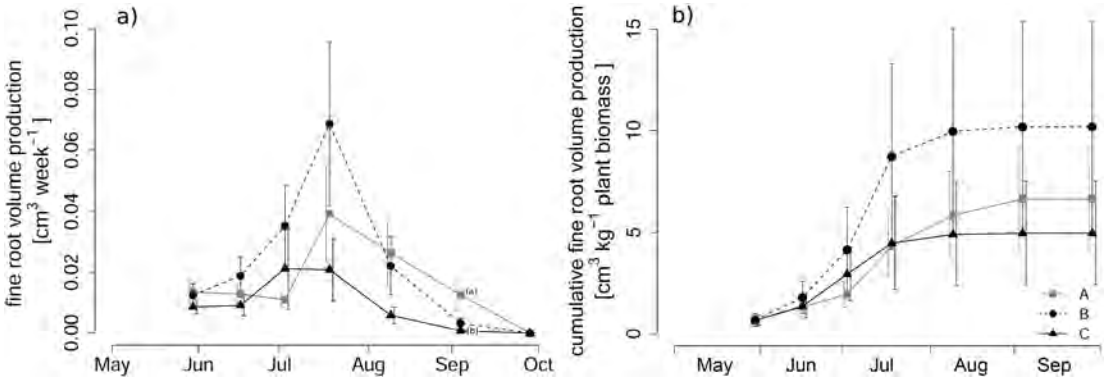

Figure 4.6: a) Fine root volume production and **b)** cumulative fine root volume production per rhizotron during the growing season of 2011 for the three treatments (mean ± SE, $n = 8$). Different letters in parentheses indicate differences between the treatments at $p < 0.1$.

Fine root NSC, root/shoot biomass ratio and fine root live/dead ratio

The fructose concentration of fine roots was positively correlated with the drought stress dose (Tab. 4.1). Concentrations of total NSC and starch also tended to increase with increasing drought stress dose, but the relationships were not statistically significant. In spite of high variability, the root/shoot biomass ratio increased with the drought stress dose. With the amount of fine root necromass being enhanced under drought, the fine root live/dead ratio was negatively correlated with the drought stress dose.

Fine root morphology

The drought treatments did not affect specific fine root length (Tab. 4.1). Fine root length distribution by fine root diameter revealed that the relative diameter class length of roots < 0.2 mm significantly increased with increasing drought stress (Fig. 4.7). Accordingly, fine roots < 0.4 mm in diameter contributed less to the total fine root length in the drought treatments. Specific root tip density was not significantly correlated with drought stress (Tab. 4.1).

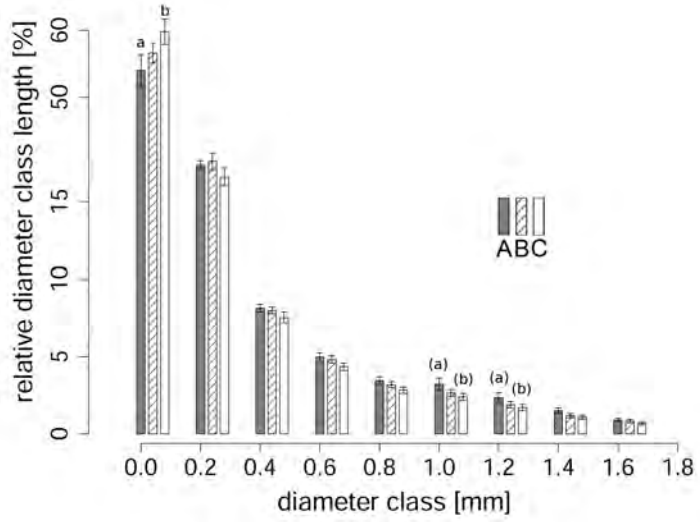

Figure 4.7: Fine root diameter class lengths of the beech fine roots for the three treatments after harvest in autumn 2010 (mean $\pm$ SE, $n = 8$). Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$.

Table 4.1: Means of plant parameters of the beech saplings after harvest 2010 for the three treatments (standard error in parentheses) and results of the correlation analysis with individual drought stress dose. Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$. Coefficient of determination (r^2) and p-value of the slope (p) are obtained from linear regression, ρ denotes Spearman's correlation coefficient; n.s. = not significant

	Treatment A	**Treatment B**	**Treatment C**	$\mathbf{r^2}$	**p**	$\boldsymbol{\rho}$
plant biomass [g]	18.1 (2.28)	15.3 (2.45)	17.7 (1.8)	-	n.s.	-
total leaf area [m^2]	0.041 (0.006)	0.036 (0.006)	0.043 (0.004)	-	n.s.	-
fine root biomass [g]	3.31 (0.35)	2.61 (0.53)	2.95 (0.48)	-	n.s.	-
fine root live/dead ratio	19.11 (5.04) a(b)	6.80 (1.27) a	7.91 (1.83) (b)	0.12	0.099	-
root/shoot biomass ratio	1.59 (0.04)	1.69 (0.13)	1.81 (0.11)	0.13	0.091	0.39
specific root length [$m\,g^{-1}$]	21.9 (2.51)	24.8 (4.74)	24.1 (2.16)	-	n.s.	-
specific root tip density [10^3 g^{-1}]	8.68 (1.44)	12.52 (2.76)	13.53 (2.7)	-	n.s.	-
fine root fructose concentration [mg g^{-1}]	3.49 (0.28) a	4.36 (0.36) b	5.40 (0.34) c	0.29	0.0091	0.54
fine root starch concentration [mg g^{-1}]	35.4 (6.9)	35.9 (5.8)	52.2 (7.0)	-	n.s.	0.38
fine root NSC concentration [mg g^{-1}]	52.0 (4.5)	63.0 (7.8)	70.6 (13.8)	-	n.s.	0.50

4.5 Discussion

Drought treatment

The broad coincidence between stomatal conductance and soil water potential reflects the response of beech saplings to the differences in soil water availability. Proceeding stomata closure with decreasing water availability is a strategy to minimize transpiration and can therefore be used as an indicator of the plant's water status (Gallé and Feller, 2007). Stomatal conductance $< 0.05\,\mathrm{mol\,H_2O\,m^{-2}\,s^{-1}}$ signalizes severe drought stress of some saplings in treatment C and with delay in treatment B (Flexas et al., 2006). However, the large variability within treatment B and C indicates that some saplings were barely stressed by water deficiency. In September, similar mean stomatal conductance is attributed to the beginning of autumnal leaf senescence in all treatments.

Photosynthesis and aboveground respiration

The intra-annual dynamics of the photosynthetic rate at constant irradiance result from different stages of leaf development and the seasonal course of temperature. Maximum photosynthetic activity is achieved in July soon after leaf formation in early summer. The reduction of photosynthesis is not as strong as reported by Gallé and Feller (2007) who observed a total breakdown of photosynthesis of beech saplings under drought. We assume that high air humidity attenuated the effect of soil drought at our site.

After rewetting in August, stressed plants re-attained their initial level of photosynthesis, with the rapid recovery indicating stomatal limitation of photosynthesis (Tognetti et al., 1995). Despite low soil water potentials in August/September, photosynthesis was similar in all treatments. As mentioned above, high air humidity allowed photosynthetic CO_2 uptake on non-stress level. Later in the season, gas exchange was apparently dominated by autumnal senescence. Aboveground respiration was not affected by drought so that the ratio of respiration to photosynthesis might have increased as observed in other studies (Flexas et al., 2006; Atkin and Macherel, 2009; Rühr et al., 2009). However, our photosynthesis measurements do not provide a direct measure of C input as we cannot exclude that relative photosynthesis reduction of drought stressed saplings was stronger under higher irradiance than that used during chamber measurements.

Rhizosphere respiration

Dynamics of rhizosphere respiration followed net photosynthesis at non-limiting water availability, underlining the tight coupling between aboveground and belowground processes and the dependence of rhizosphere respiration on assimilate availability (Irvine et al., 2005; Högberg and Read, 2006). While photosynthesis of drought-stressed saplings recovered after rewetting, rhizosphere respiration

remained low and lagged behind the control for the rest of the growing season. We cannot exclude that rhizosphere respiration was under- or overestimated by subtracting soil CO_2 effluxes of planted from unplanted rhizotrons. The variation in rhizosphere respiration within the treatments is mainly triggered by differences in sapling biomass, but there is also some variation in CO_2 evolution from decomposition of soil organic matter. Despite the methodological uncertainty, we conclude that a greater proportion of the assimilated C was translocated to the root system with increasing drought stress dose and that rhizosphere respiration was rather limited by competing C sinks than by decreased C assimilation. Furthermore, our method does not distinguish between microbial respiration and root respiration which might respond differently to drought. The observed pattern could therefore be attributed to a decline in root respiration or heterotrophic respiration of microorganisms which rely on substrate transfer from roots.

Fine root production

As rhizotron images only display visible roots on transparent side walls, an extrapolation of fine root production to the whole root system is difficult (Joslin et al., 2000). Presuming similar initial fine root biomass prior to the soil water manipulation, we interpret the unimodal response of fine root production as a drought effect. The promotion of fine root growth at moderate drought is in accordance with other studies (e.g. Leuschner et al., 2001) and is understood as a strategy to improve water uptake. Such a response was absent under severe drought with reduced fine root production. We explain this by reduced assimilate availability corresponding to the decline in rhizosphere respiration. Furthermore, increased physical soil resistance at severe drought is assumed to limit root growth (Bengough et al., 2006). Root growth of loblolly pine has been reported to cease between -0.3 and -1.2 MPa (Torreano and Morris, 1998) which corresponds to the moisture range between treatment B and C in our experiment. However, the effect of soil density on root growth is not only soil-specific, but also species-specific (Siegel-Issem et al., 2005). We did not observe compensatory root growth during severe soil drought but we can not exclude that rewetting would have promoted root growth after a certain recovery time.

Root/shoot biomass ratio, fine root live/dead ratio and NSC

Increasing concentration of fructose in the fine roots is interpreted as a response to soil drought as this sugar lowers the osmotic potential in the plant as a prerequisite of enhanced water uptake (Kameli and Lösel, 1993). Although not significantly correlated, accumulation of total NSC as well as the enhanced proportion of starch perhaps reflected restricted assimilate investment into tissue growth, accompanied by decoupling of rhizospheric respiration from photosynthesis (Irvine et al., 2005).

However, the increase of the root/shoot biomass ratio with increasing drought stress dose indicates that, in relative terms, C allocated to the belowground compartments was yet rather invested for biomass increment than for respiration. We could not directly calculate fine root turnover from the repeated root observations due to the lack of dead roots on the rhizotron side walls. Fine root live/dead-ratio has been discussed to serve as a proxy for this parameter (Godbold et al., 2003; Zang et al., 2011). The decrease in the live/dead-ratio in treatment B and C resulted from drought-induced fine root dieback (c.f. Gaul et al., 2008; Leuschner et al., 2001). Root necromass, on the other hand, is also controlled by root decomposition which is retarded under drought (Gaul et al., 2008). We can therefore not definitely conclude that fine root turnover was accelerated under drought.

Fine root morphology

There was no marked effect of drought on fine root morphology. Nevertheless, a tendency towards an increased proportion of fine roots < 0.2 mm is interpreted as a strategy to enhance the root surface area per unit of C investment. As drought was limited to two month in our experiment, a rapid response of fine roots becomes apparent. Assuming that a longer period of drought would have led to more pronounced results, we corroborate other studies showing a particular plasticity of the fine root system of European beech (Meier and Leuschner, 2008b).

4.6 Conclusions

Planted beech saplings were sensitive to drought stress. Photosynthesis was less affected than rhizosphere respiration indicating a shift in assimilate utilization under drought. As an instantaneous response, fine root growth was promoted at moderate soil drought, but decreased at severe drought. Even upon incipient drought, increase of belowground C allocation and fine root mortality became apparent. Morphological fine root parameters indicated enhanced effectiveness in soil moisture exploitation under drought.
The results of our study refer to the status quo after the drought treatment. We can not exclude compensatory effects after rewetting and in the subsequent growing seasons as described by Olesinski et al. (2011). However, this study gives useful information on the behavior of planted beech saplings upon soil drought and provides a reference for drought stress quantification in future experiments.

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5 Fate of recently fixed carbon in European beech (*Fagus sylvatica* L.) saplings during drought and subsequent recovery

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5.1 Abstract

Drought reduces the carbon (C) assimilation of trees and decouples aboveground from belowground carbon fluxes but little is known about the response of drought-stressed trees to rewetting. This study aims to assess dynamics and patterns of C allocation in beech saplings under dry and rewetted soil conditions.
In October 2010, five-year-old beech saplings from a forest site were transplanted into 20 l pots. In 2011, the saplings were subjected to different levels of soil drought between non-limiting water supply (control) to severe water limitation with soil water potentials of less than -1.5 MPa. As a physiologically relevant measure of drought, the cumulated soil water potential (i.e., drought stress dose) was calculated for the growing season. In late August, the saplings were transferred into a climate chamber and pulse-labeled with ^{13}C-depleted CO_2 (δ^{13}C of -47 ‰). Isotopic signatures in leaf and soil respiration were repeatedly measured. Five days after soil rewetting, a second label was applied using 99 atom-% $^{13}CO_2$. After another 12 days, the fate of assimilated C in each sapling was assessed by calculating the ^{13}C mass balance.
Photosynthesis decreased by 60 % in saplings under severe drought. The mean residence time of recent assimilates in leaf respiration was more than three times longer than under non-limited conditions and was positively correlated to drought stress dose. Also the appearance of label in soil respiration was delayed. Within five days after rewetting, photosynthesis, mean residence time of recent assimilates in leaf respiration and appearance of label in soil respiration fully recovered. Despite the fast recovery, less label was recovered in the biomass of the previously drought-stressed plants which also allocated less C to the root compartment (45 % vs. 64 % in the control).
We conclude that beech saplings quickly recover from extreme soil drought, although transitional after-effects prevail in C allocation, possibly due to repair-driven respiratory processes.

5.2 Introduction

A key parameter for understanding the carbon (C) turnover in terrestrial ecosystems is the aboveground and belowground C allocation of plants (Horwath et al., 1994; Trumbore, 2006). Up to 60 % of soil respiration has been shown to be directly fueled by recently assimilated C and there is a tight temporal coupling between aboveground and belowground C fluxes (Steinmann et al., 2004; Högberg and Read, 2006). Changes in C allocation can therefore affect the C sequestration of ecosystems (Trumbore, 2006; Carbone et al., 2007).
Drought is expected to become an increasingly important climatic stressor in many regions of the earth (IPCC, 2007), not only influencing physiological plant

parameters such as photosynthesis and, hence, ecosystem-level gross primary production (Granier et al., 2007; Ciais et al., 2005), but also patterns and dynamics in plant C allocation. The tight temporal coupling of aboveground and belowground C fluxes is impaired by drought (Bréda et al., 2006; Kuzyakov and Gavrichkova, 2010; Barthel et al., 2011; Dannoura et al., 2011), as evidenced by increasing mean residence times (MRTs) of recently formed assimilates in different plant compartments (Rühr et al., 2009).

European beech (*Fagus sylvatica* L.) is an ecologically dominant tree species in Central Europe of high economic importance. Known to be drought-sensitive (Backes and Leuschner, 2000; Gessler et al., 2004, 2006; Michelot et al., 2012), especially during early stages of establishment (Fotelli et al., 2001; Lendzion and Leuschner, 2008), European beech has been reported to recover quickly from drought stress and to survive severe drought episodes (Gallé and Feller, 2007). Such findings gave rise to controversial debates about the silvicultural consequences of climate change for European beech in Central Europe (e.g. Rennenberg et al., 2004; Ammer et al., 2005). Tognetti et al. (1995) observed a recovery of photosynthesis, leaf water potential and chlorophyll concentration of drought-stressed beech seedlings from two Italian populations within 5 days after rewetting, whereas leaf conductance did not fully recover during this period. Similar findings are reported by Gallé and Feller (2007) for beech saplings, although complete recovery of photosynthesis required 4 weeks. However, allocation dynamics of recently formed photoassimilates upon different levels of drought and subsequent rewetting remain obscure.

Comparability of studies concerning soil drought has often been restricted by the lack of stress quantification, given that most often volumetric soil water content was considered, which does not reflect soil water availability (Vicca et al., 2012). Stomatal closure per se is questionable as a drought indicator, as beech provenances can differ in stomatal sensitivity (Peuke et al., 2002; Rose et al., 2009). In our integrated field and laboratory experiment, we made use of the soil water potential as a physiologically relevant measure of drought stress and employed the cumulated soil water potential (referred to as the drought stress dose (DSD)) as an explanatory variable (Zang et al., 2013). In doing so, we subjected planted beech saplings from a reforestation site to defined levels of drought stress and subsequent rewetting. Twofold ^{13}C labeling, before and after rewetting, was applied for every plant, allowing for the calculation of an individual C balance.

We hypothesized that increasing drought stress impedes the C translocation to the belowground plant compartments as reflected by increased MRTs of recently formed photosynthates in leaves and delayed appearance in soil respiration. We further hypothesized that, after rewetting, effects of drought on these parameters and C partitioning prevail, depending on the intensity of the preceding drought stress.

5.3 Materials and Methods

Study site and experimental setup

Two-year-old beech saplings of a local provenance were planted in a mature Norway spruce forest (tree age 145 years) in the Fichtelgebirge, Bavaria, Germany (50° 8' N, 11° 52' E), in autumn 2008 (see GERSTBERGER et al. (2004) and SCHULZE et al. (2009) for detailed site description). The saplings were of a local provenance and were bare rooted. After two growing seasons with optimum soil water availability, 36 randomly chosen beech saplings (for leaf area, see Tab. 5.1) were excavated including the rooted soil monolith and transferred into plastic pots (diameter: 29.5 cm, height: 32 cm) that were perforated at the bottom to allow water drainage. The organic layer, which contained a large amount of herbaceous roots, was omitted and replaced by a sand layer with a thickness of 5 cm. Each pot was equipped with a FDR (frequency domain reflectometry) soil moisture sensor (EC-20, Decagon Devices, Pullman, WA, USA), which was installed vertically to integrate volumetric soil water content (VWC) from 10 to 30 cm soil depth. The pots were subsequently embedded into the surrounding soil at the forest site to maintain a natural vertical temperature gradient.

Drought treatment and drought stress quantification

Prior to measurements, the plants had been randomly assigned to three groups of differing soil water availability, representing non-limited soil water availability (control = Cont), moderate drought (mD) and severe drought (sD) as corresponding to mean target soil water potentials of -0.05 MPa, -0.6 MPa and -1.2 MPa, respectively.

A translucent roof construction (height: 2.2 m) was installed above the potted saplings in late June 2011 to exclude throughfall. Soil water potential was measured in the soil of every pot 5 to 15 times during the duration of the experiment using a tensiometer for the moisture range greater than -0.3 MPa (T5 tensiometer, UMS, Munich, Germany) and a dewpoint potentiometer for soil water potentials less than -0.3 MPa (WP4 dewpoint potentiometer, Decagon Devices, Pullman, WA, USA). Adjustment of the respective target soil water potential was conducted by individual irrigation with deionized water via perforated plastic containers that were brought in direct contact to the mineral soil. This way of water application assured slow infiltration and homogeneous distribution of water within in the soil. We correlated measured soil water potentials with the corresponding FDR sensor signal and fitted individual spline regression functions. These were used to model the time course of soil water potential based on the hourly logged FDR sensor signal. The drought stress dose for individual beech saplings was defined as the cumulated soil water potential during the growing season, i.e.,

$$DSD = -\int \Psi\,(t)\; dt \qquad (1)$$

where *DSD* is the drought stress dose [MPa day] and $\Psi(t)$ is the individual time course of soil water potential as modeled within the time period between bud burst and harvest [MPa].

First pulse label

The pots were removed from the surrounding soil and transported to a climate chamber at the University of Bayreuth on 18 August 2011. Air temperature was held constant at 18 °C and relative humidity at 80 %. A light source providing photosynthetically active radiation (PAR) of a photon flux density of 500 μmol m^{-2} s^{-1} at shoot apex height was employed for 16 h a day (MT400DL/ BH-E40, Iwasaki Electric, Japan). The pots were arranged randomly inside the chamber and moved regularly during the experiment. Atmospheric air was continually passed through the chamber providing a full air exchange every 1.5 h.
A first pulse label with ^{13}C-depleted CO_2 was applied individually around noon from August 30^{th} through September 2^{nd}. For this purpose, a quadratic plate (polyvinylchloride, 45 x 45 cm, 1 cm thick) with a notch was fitted around the beech stems. With its gasket on the bottom side, the plate rested flat on the container rim. The notch was made air tight with a sealant (Terostat, Henkel, Düsseldorf, Germany). A translucent labeling chamber (44 × 44 cm, height 130 cm, volume = 250 l, polycarbonate) was placed over each sapling and tightly fixed on the plate. A fan inside the chambers ensured air mixing. Diffusive CO_2 loss from chambers had been quantified as < 7 ppm per hour for a CO_2 concentration gradient between chamber air and atmosphere of ~ 600 ppm. Due to heating by the light source, the chamber air was warmer than the ambient air but stabilized at 21±1 °C after ~ 1 h.
Thirty plants (n = 10 per treatment) were labeled during three sessions, irrespective of the drought treatment, on three consecutive days, whereas 6 plants (n = 2 per treatment) served as unlabeled controls. Labeling started between 9:00 am and 11:00 am (labeling times see Tab. 5.1). The target value of the CO_2 concentration inside the chambers was between 500 and 1000 ppm during the labeling period. To achieve this aim, we measured photosynthetic CO_2 uptake of each plant within one chamber instalment prior to labeling using a infra-red gas analyser (LiCor 820, Licor Inc., Lincoln, NE, USA, air flow rate: 1 l min^{-1}). Based on these measurements, the frequency of label injections was calculated to sustain the target CO_2 concentration range during the labeling period. Labeling gas (100 % CO_2, δ^{13}C = -47 ‰, DIN EN ISO 14175:C1, Westfalen AG, Münster, Germany) was injected with a gas-tight syringe with a maximum amount of 120 ml CO_2 per injection. Upon removal of the labeling chambers, the climate chamber was immediately flushed with atmospheric air to attenuate remaining label and prevent contamination of other plants.

CO_2 from leaf respiration was sampled for isotope-ratio mass spectrometry (IRMS) immediately before labeling (natural abundance) and at 20, 29, 44, 54, 73, 97 and 121 h after termination of labeling. To this end, on each plant a non-transparent PTFE-coated gas bag (volume of 0.5 l) was tightly fitted around one lateral branch with ~10 leaves. Gas bags were sealed and then flushed with CO_2-free air. Gas samples were taken through a septum after 30 min for isotopic assessment of recently respired C. An individual exponential model was fitted to the time course of the isotopic signature of leaf respiration for each beech sapling as follows:

$$atom\%^{13}C_{t1} = atom\%^{13}C_{t0} \times e^{-kt} - atom\%^{13}C_{NA} \qquad (2)$$

where $atom\%^{13}C_{t1}$ is atom percent of ^{13}C of leaf respiration at time *t1*, $atom\%^{13}C_{t0}$ is initial atom percent of ^{13}C of leaf respiration, k is the fitted decay constant [h^{-1}], t is the time after labeling [h] and $atom\%^{13}C_{NA}$ = atom percent of ^{13}C of leaf respiration before labeling.
The mean residence time (MRT) of label in leaf respiration was calculated as follows:

$$\mathrm{MRT} = 1/\mathrm{k} \qquad (3)$$

To measure the rate of soil respiration and its δ^{13}C, the bottom plates of the labeling chambers were put on the pots and made air-tight with sealant (Terostat). The increase of the CO_2 concentration in the headspace was monitored (IRGA) during an incubation time of 4 min (flow rate: 0.5 L min^{-1}). The respiration rate was calculated from the slope of the linear regression between CO_2 concentration and incubation time considering the individual headspace volume according to Borken et al. (2006).
Subsequently, the headspace was flushed with CO_2-free synthetic air until no CO_2 could be detected. This procedure was repeated three times. For ^{13}C isotope analyses of soil respiration, the soil was subsequently incubated with the incubation time depending on soil respiration rate in order to obtain a headspace CO_2 concentration of ~1000 ppm. Again, gas samples were taken through the septum with a syringe and stored in 5 ml glass vials (Exetainer, Labco Limited, Buckinghamshire, UK) before being analyzed within 7 days for isotopic signature (GVI-Isoprime, Elementar Analysensysteme GmbH, Hanau, Germany). Soil respiration was sampled on seven time points: immediately before the labeling and at 29, 44, 54, 73, 97 and 143 h after termination of labeling.

Rewetting

Six days after the first pulse label, all saplings were irrigated to a target soil water potential of -0.05 MPa. Deionized water was applied by small portions within five hours. Maximum irrigation per plant was 3.2 l which corresponded to a precipitation event of 45 mm.

Second pulse label and ^{13}C mass balance

A second pulse label was applied five days after rewetting with enriched $^{13}CO_2$ (99 atom-% ^{13}C, Eurisotop, Saint-Aubin, France). The procedure was conducted in analogy to the first labeling. Additionally, gas samples from each chamber were taken before the label application as well as at the end of the labeling period and analyzed for δ^{13}C and CO_2 concentration. The amount of ^{13}C taken up by each plant ($m^{13}C_{uptake}$) was calculated as follows:

$$m^{13}C_{uptake} = m^{13}C_{t1} - m^{13}C_{t2} + m^{13}C_{injected} \qquad (4)$$

where $m^{13}C_{t1}$ is the amount of ^{13}C in chamber air before labeling, $m^{13}C_{t2}$ correspondingly after labeling and $m^{13}C_{injected}$ the respective amount injected during labeling.

We calculated a ^{13}C mass balance for every beech sapling at day 12 after application of the second label as follows:

$$m^{13}C_{uptake} = m^{13}C_{AR} + m^{13}C_{SR} + m^{13}C_{Bio} + m^{13}C_{S} \qquad (5)$$

where $m^{13}C_{AR}$ is the amount of ^{13}C emitted by aboveground respiration (mainly leaf respiration ($m^{13}C_{LR}$), see below), $m^{13}C_{SR}$ emitted by soil respiration, $m^{13}C_{Bio}$ recovered in plant biomass and $m^{13}C_{S}$ remaining in the soil solid phase. To consider solely label-derived ^{13}C in the specific compartments, atom% excess (APE) was calculated relative to the isotopic signature of the corresponding unlabeled control plants which had been subjected to the same drought treatments as follows:

$$APE = atom\%_{Sample} - atom\%_{NA} \qquad (6)$$

where $atom\%_{Sample}$ = atom percent of ^{13}C of the sample after labeling and $atom\%_{NA}$ = atom percent of ^{13}C in unlabeled control plants (natural abundance). Abundance of ^{13}C of leaf respiration was measured at 20, 30, 45, 51, 73, 99, 121 and 296 h after termination of labeling. Light and dark-adapted leaf respiration rates were determined on a single-leaf basis with a portable CO_2-H_2O porometer equipped with an infrared gas analyzer (LiCor 6400, Licor Inc., Lincoln, NE, USA) and a cuvette providing a red-blue LED light source. Total leaf area was determined after harvest (see below). At two occasions, light and dark-adapted respiration rate of the total aboveground plant compartment were assessed by measuring the CO_2 accumulation rate in the labeling chambers in the absence of light. We found that foliage respiration extrapolated from single-leaf measurements accounted on average for 91±4.7 (SD) % of total aboveground respiration. We calculated the label-derived ^{13}C in leaf respiration (both light and dark-adapted respiration) ($m^{13}C_{LR}$) within the observed time span as follows:

$$m^{13}C_{LR} = \int F_{LR} \times APE_{LR}(t)/100\, dt \qquad (7)$$

where F_{LR} is the mean of light and dark-adapted leaf respiration rate, weighted by the set day length of 16 h in the climate chamber and $APE_{LR}(t)$ is the atom percent excess of leaf respiration at time t.
Isotopic signature of CO_2 in soil respiration as well as soil respiration rates were measured at 10 time points: 0, 7, 20, 30, 45, 51, 73, 99, 146 and 296 h after termination of labeling. The amount of label-derived ^{13}C emitted from the soil within 12 days after labeling was calculated as follows:

$$m^{13}C_{SR} = \int F_{SR}(t) \times APE_{SR}(t)/100\,dt \qquad (8)$$

where $F_{SR}(t)$ is the soil respiration rate at time t and $APE_{SR}(t)$ is the atom percent excess of soil respiration at time t.

Beech saplings were clipped and separated into leaves, buds, twigs (shoot parts < 2 mm in diameter) and stem (shoot parts > 2 mm in diameter) 296 h after labeling. Leaf area was determined through digital image evaluation (SigmaScan 5, Systat Software Inc., Chicago, IL, USA) after foliage scanning. Six vertical soil cores along the entire soil profile (diameter = 2 cm) were randomly taken from each pot, homogenized, separated from visible root fragments and stored at -22 °C until further analyses. Samples from fine (< 2 mm) and coarse roots were taken. During the following 4 weeks, the amount of live fine roots and coarse roots as well as the soil volume were quantified in each pot. All plant compartments were weighed and ground with liquid nitrogen before isotopic analyses (vario MAX, Elementar Analysensysteme, Hanau, Germany). All plant material was oven dried at 60 °C until constant weight was achieved. The label-derived amount of ^{13}C in total plant biomass ($m^{13}C_{Bio}$) was then calculated as follows:

$$m^{13}C_{Bio} = \Sigma\ m_i \times \%C_i/100 \times APE_i/100 \qquad (9)$$

where m_i is the dry mass, $\%C_i$ the carbon content [%] and APE_i the atom percent excess of ^{13}C of each plant compartment.
Owing to the considerable background of organic C in the soil (1-2 % C), $m^{13}C_S$ was estimated by assessing the isotopic signature of hot water extractable soil organic carbon (SOC) , which was expected to serve as a proxy for soluble carbohydrates originating from root exudates, microbial biomass or small root fractions like root hairs. For that purpose, deionized water was added to a subsample of the soil (20 g) that had been frozen directly after sampling (soil:solute mass ratio = 1:5). The soil was extracted for 24 h at 70 °C. The supernatant was filtered (0.45 μm) and freeze-dried. The residual fraction was homogenized and analyzed for its non-purgeable organic C content with an elemental analyzer (multi N/C 2100, Analytik Jena, Jena, Germany) and its isotopic signature with an isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany, coupled

to the elemental analyzer NA 1108, CE Instruments, Hindley Green, UK). The amount of label-derived hot water soluble organic ^{13}C was calculated for each pot in relation to individual soil volumes.

Statistical analyses

Differences between the treatments were analysed using Tukey's HSD test after analysis of variance (n = 10); normality was assumed when data passed the Shapiro-Wilk-test ($p > 0.1$). In the case of non-normally distributed data, the non-parametric Wilcoxon test was conducted followed by the Kruskal-Wallis analysis of variance. Additionally, assessed plant parameters were subjected to a linear regression with the individual DSD; the relationship was then characterized by the p-value of the slope as well as by the adjusted r^2 and Spearman's correlation coefficient. The DSD as the explaining variable was log-transformed ($\log$ DSD) before regression analysis. All calculations involving ^{13}C abundance (calculation of the mean and standard deviation, statistic tests, regression analyses) were performed after transformation of δ‰ values to atom% or APE. Results were also displayed in the common delta-notation for clarity. All statistical analyses were performed using R 2.13.0 (R Development Core Team, 2009).

5.4 Results

Time course of soil water potential during the experiment

Maximum drought in treatments mD and sD was achieved 6 weeks after exclusion of precipitation. Mean soil water potential dropped to -0.82 MPa and -1.4 MPa, respectively, at that time (Fig. 5.1). Minimum soil water potential of -3.0 MPa occurred in treatment sD. Soil water potential in the control ranged between -0.03 and -0.06 MPa. After rewetting, a target soil water potential of -0.05 MPa was achieved within 1 day in treatments mD and sD. Thereafter, soil water potential remained above -0.06 MPa irrespective of treatment.

Plant parameters before rewetting

Photosynthesis significantly declined with decreasing soil water potential. Immediately before the first labeling, mean net photosynthesis rate was 7.0±1.1 (SD) μmol CO_2 m^{-2} s^{-1} in the control, which was reduced to 2.8±3.0 μmol CO_2 m^{-2} s^{-1} in sD (Tab. 5.2). The negative correlation between photosynthesis and the individual DSD was highly significant.

Natural abundance of ^{13}C in leaf and soil respiration of control plants was significantly lower than of the stressed plants (Tab. 5.2), resulting in a significant positive correlation with DSD (Tab. 5.2, Fig. 5.2).

The application of ^{13}C-depleted CO_2 during the first labeling pulse caused a decrease of the isotopic signature of leaf respiration (Fig. 5.2), its shift relative to nat-

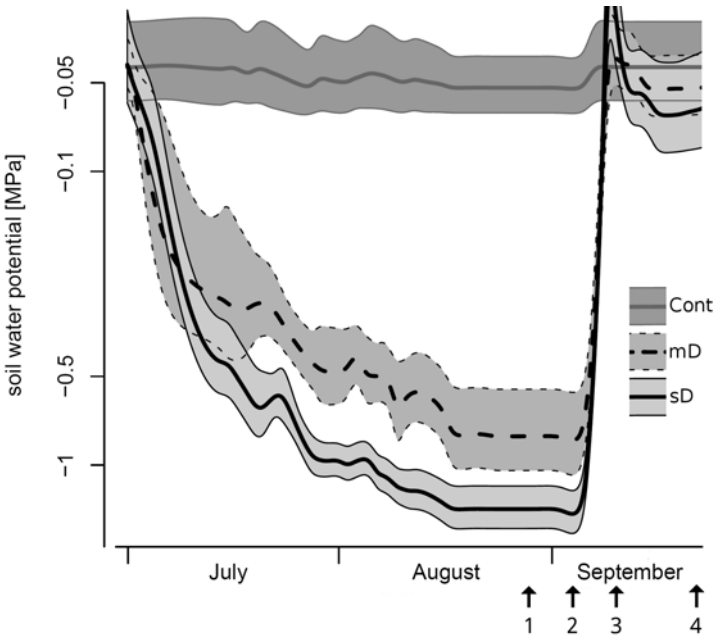

Figure 5.1: Time course of soil water potential for the three treatments (Cont = control, mD = moderate drought, sD = severe drought), n = 10, mean±SE. Arrows indicate the time point of the first pulse label (1), rewetting (2), second pulse label (3) and harvest (4).

ural abundance declined over time. The MRT of the label in leaf respiration was more than three times longer at sD than in the control (107 h vs. 30 h). Furthermore, MRT was positively correlated with DSD ($p = 0.014$, Tab. 5.2, Fig. 5.5 a). Minimum $\delta^{13}C$ in soil respiration was observed at the first sampling date (29 h after the labeling) under the control and mD. In contrast, sD achieved its minimum ^{13}C abundance at the second sampling date at 44 h after labeling (Fig. 5.3).

Plant parameters after rewetting

Rewetting induced fast recovery of photosynthesis within 3 days (Tab. 5.2). Before the second pulse labeling, differences in net photosynthesis between the treatments had vanished, so that correlation with DSD did not exist anymore. After second labeling, $\delta^{13}C$ of leaf respiration rose to up to > 4000 ‰ in any treatment before declining exponentially (Fig. 5.4). Mean MRT extended through ~ 50 h irrespective of treatment, indicating absence of persisting drought effects upon rewetting (Tab. 5.2, Fig. 5.5). The ^{13}C peak in soil respiration occurred between 30 and 51 hours after labeling in any treatment (Fig. 5.4) and in the absence of treatment effects.

Table 5.1: Plant characterization and details of the labeling procedure for the three treatments (Cont = control, mD = moderate drought, sD = severe drought). Plant parameters were assessed after harvest (means, standard deviation in parentheses).

	Cont	**mD**	**sD**
total plant biomass [g]	135 (28)	124 (28)	119 (27)
root/shoot ratio	0.91 (0.13)	0.83 (0.23)	0.86 (0.17)
leaf area [m^2]	0.256 (0.084)	0.245 (0.067)	0.258 (0.095)
labeling time (first labeling) [min]	412 (46)	421 (41)	441 (35)
CO_2 uptake during first labeling [mmol]	42.6 (11.6)	22.3 (8.6)	13.9 (6.9)
labeling time (second labeling) [min]	247 (40)	259 (41)	255 (35)
CO_2 uptake during second labeling [mmol]	19.7 (5.3)	17.5 (6.2)	17.0 (5.2)

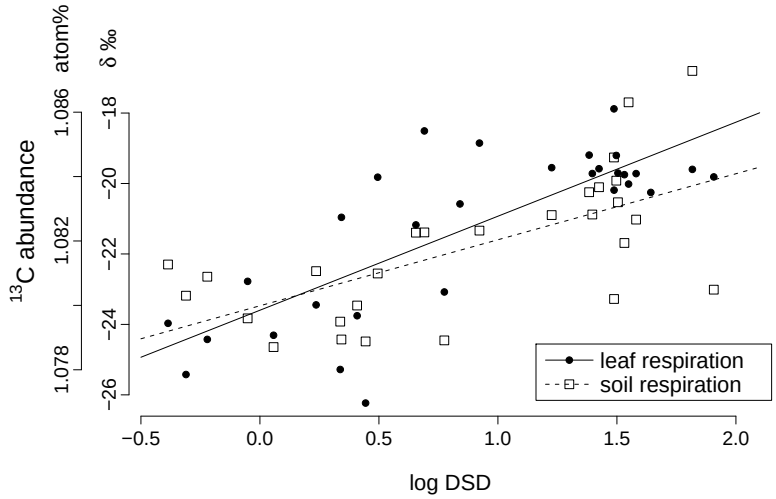

Figure 5.2: Correlation between DSD (drought stress dose) and natural abundance of ^{13}C in leaf respiration and soil respiration immediately before the first pulse label. Statistical information see Tab. 5.2.

Table 5.2: Overview of different plant parameters for the three treatments (Cont = control, mD = moderate drought, sD = severe drought) before and after rewetting (means, standard deviation in parentheses). The greek letters represent p-values yielded from multiple comparison analysis with α = difference between Cont and mD, β = difference between Cont and sD and γ = difference between mD and sD. Results of the regression analyses with DSD (drought stress dose) are represented by p (p-value of the slope), r^2 (adjusted coefficient of determination) and ρ (Spearman's correlation coefficient). n.a. = natural abundance

		Cont	**mD**	**sD**	$\boldsymbol{\alpha}$	$\boldsymbol{\beta}$	$\boldsymbol{\gamma}$	**p**	$\mathbf{r^2}$	$\boldsymbol{\rho}$
before rewetting	n. a. of ^{13}C in leaf respiration [‰]	-24.3 (1.1)	-19.7 (0.8)	-19.6 (0.8)	<0.001	<0.001	0.977	<0.001	0.61	0.66
	n. a. of ^{13}C in soil respiration [‰]	-23.4 (1.0)	-21.5 (0.8)	-20.4 (2.5)	0.018	<0.001	0.327	<0.001	0.42	0.63
	photosynthesis rate [μmol CO_2 m^{-2} s^{-1}]	7.0 (1.1)	3.6 (1.6)	2.8 (3.0)	0.005	<0.001	0.653	<0.001	0.53	-0.75
	MRT of label-derived ^{13}C in leaf respiration [h]	30 (12)	94 (39)	107 (58)	0.007	0.002	0.774	0.014	0.20	0.50
after rewetting	photosynthesis rate [μmol CO_2 m^{-2} s^{-1}]	7.0 (0.8)	6.4 (1.6)	6.5 (1.8)	0.673	0.727	0.995	0.466	0.02	-0.07
	MRT of label-derived ^{13}C in leaf respiration [h]	51 (19)	48 (28)	51 (18)	0.95	0.99	0.96	0.695	0.00	-0.06
	% of ^{13}C uptake recovered in:									
	hot water soluble SOC	11.6 (9.4)	10.8 (9.0)	13.7 (13.6)	0.980	0.903	0.827	0.116	0.09	0.31
	soil respiration	13.2 (4.4)	12.0 (4.0)	12.2 (6.7)	0.865	0.913	0.991	0.499	0.02	-0.17
	leaf respiration	16.9 (4.6)	24.8 (11.8)	24.2 (11.7)	0.189	0.243	0.988	0.199	0.06	0.20
	plant biomass	41.1 (14.0)	39.8 (11.9)	30.9 (3.8)	0.959	0.106	0.176	0.009	0.22	-0.42
	thereof									
	leaves	5.0 (2.8)	8.0 (4.9)	8.1 (4.9)	0.275	0.255	0.987	0.114	0.09	0.33
	buds	1.9 (0.7)	2.4 (1.5)	2.5 (1.6)	0.641	0.525	0.980	0.475	0.02	0.06
	twigs	1.7 (1.0)	2.3 (1.4)	2.2 (1.1)	0.472	0.574	0.984	0.351	0.03	0.10
	stem	5.6 (4.0)	5.9 (5.0)	4.5 (2.3)	0.987	0.802	0.714	0.428	0.02	-0.21
	coarse roots	13.6 (11.0)	8.2 (10.1)	5.5 (3.0)	0.365	0.116	0.775	0.007	0.23	-0.38
	fine roots	13.3 (8.2)	13.0 (7.4)	8.0 (4.2)	0.992	0.208	0.253	0.111	0.08	-0.20
	relative to label-derived ^{13}C in plant biomass									
	leaves	14.2 (11.1)	22.8 (16.0)	25.9 (14.4)	0.362	0.165	0.877	0.047	0.15	0.43
	buds	4.8 (1.2)	6.5 (4.1)	8.0 (4.3)	0.519	0.109	0.589	0.101	0.09	0.42
	twigs	4.0 (1.9)	5.7 (3.1)	7.1 (3.3)	0.402	0.058	0.525	0.044	0.14	0.36
	stem	13.5 (7.7)	13.3 (8.9)	14.4 (7.0)	0.990	0.960	0.950	0.928	0.00	-0.11
	coarse roots	29.7 (16.5)	18.5 (15.0)	18.1 (9.7)	0.194	0.175	0.997	0.021	0.20	-0.31
	fine roots	33.9 (17.9)	33.2 (16.6)	26.5 (14.7)	0.990	0.585	0.638	0.699	0.01	0.04

Overall, between 81 and 88 % of the applied ^{13}C was recovered in leaf respiration, soil respiration, plant biomass and hot water soluble SOC irrespective of treatment (Tab. 5.2, Fig. 5.6). About 12-13 % of the applied ^{13}C were released in general by soil respiration and 11-14 % recovered as hot water soluble C. Under sD, less ^{13}C was incorporated into the living biomass of plants (31 % of applied ^{13}C) than under the other treatments (41 and 40 %) in Cont and mD, respectively; Fig. 5.6).
Consistently, the incorporation of ^{13}C in plant biomass was negatively and significantly correlated with DSD. Previously drought-stressed plants tended to exhibit higher ^{13}C release via leaf respiration than non-stressed plants (Fig. 5.7). With increasing DSD, the proportion of label-derived ^{13}C recovered in leaves and twigs became significantly enhanced. Conversely, ^{13}C in coarse roots significantly decreased with increasing DSD. Overall, 64, 52 and 45 % of biomass-bound ^{13}C were recovered in roots under Cont, mD and sD, respectively.

5.5 Discussion

a) Drought effects

Natural abundance and photosynthesis

Natural abundance of ^{13}C in leaf and soil respiration is in accordance with the 60 % reduction of photosynthesis under drought conditions. Stomatal closure leads to a reduced discrimination of ^{13}C and therefore increases the isotopic signature of assimilates (Dawson et al., 2002). Thus, the observed pattern underpins the physiological response of beech saplings to soil water availability in our experiment. Furthermore, the significant correlations of natural abundance of ^{13}C both in leaf and soil respiration with the individual drought stress dose highlights the physiological relevance of cumulated soil water potential with respect to stress quantification.
However, as DSD summarizes water availability of several months it does not necessarily reflect single drought events in terms of their intensity and duration. In our study the individual soil water potential was monotonically lowered until the target level was reached, except for small fluctuations due to the irrigation routine. Hence, DSD correlated with the minimum soil water potential within our sample collective, effectively reflecting drought exposure at the single-tree level. The introduced concept of drought stress quantification needs to be validated at forest sites that differ in drought and precipitation patterns. Threshold ranges of drought stress may be implemented as discussed by Vicca et al. (2012), e.g. in analogy to Granier et al. (2007) who defined water stress based on a threshold value of relative extractable water.

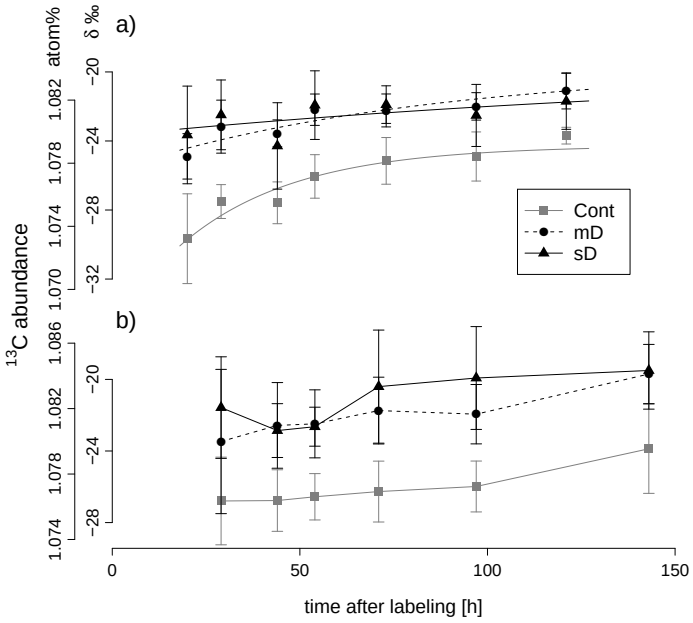

Figure 5.3: ^{13}C abundance following the first pulse label (before rewetting, δ^{13}C of labeling gas = -47 ‰) in leaf respiration **(a)** and soil respiration **(b)** for the three treatments (Cont = control, mD = moderate drought, sD = severe drought), n = 10, mean±SD. Statistical information and mean residence times see Tab. 5.2.

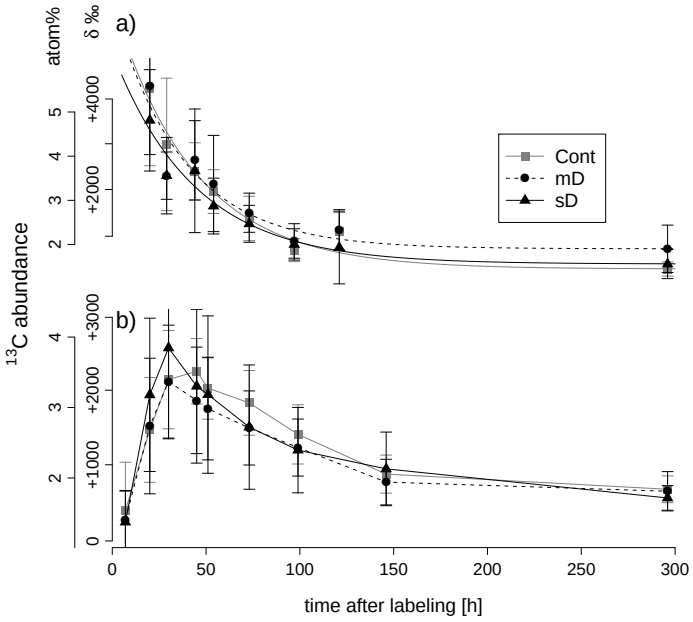

Figure 5.4: ^{13}C abundance following the second pulse label (after rewetting, ^{13}C abundance in the labeling gas = 99 atom%) in leaf respiration **(a)** and soil respiration **(b)** for the three treatments (Cont = control, mD = moderate drought, sD = severe drought), n = 10, mean±SD. Statistical information and mean residence times see Tab. 5.2.

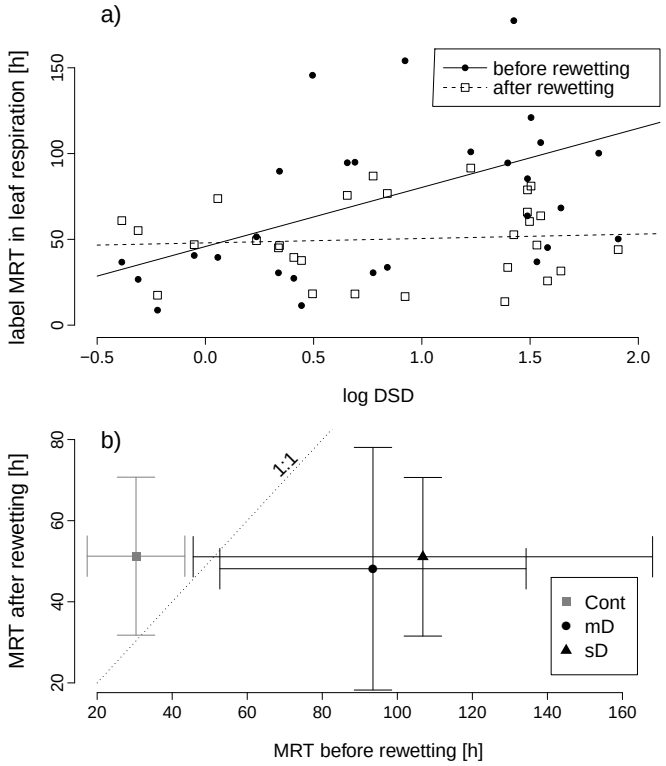

Figure 5.5: (a) Regression of DSD (drought stress dose) on MRT (mean residence time) of label-derived ^{13}C in leaf respiration before and after rewetting. Statistical information see Tab. 5.2. **(b)** Difference in MRT before and after rewetting for the three treatments (Cont = control, mD = moderate drought, sD = severe drought).

MRT of label in leaf respiration and label appearance in soil respiration

Drought increased MRT of the label in leaf respiration, indicating prolonged retention of recently formed assimilates. RÜHR et al. (2009) reported on a doubled mean residence time of excess ^{13}C in leaf water-soluble organic matter of drought-stressed beech saplings. This prolonged assimilate retention in the mesophyll may be associated with tissue dehydration and reduction in phloem loading due to reduced carbohydrate production rather than with increased emission of biogenic volatile organic compounds (BVOC) or changes in the leaf respiration rate. Given the about three times higher MRT of recently formed assimilates in sD than in control leaves, we conclude that the effective maximum drought was stronger in our experiment. Regarding the significant positive correlation between MRT of label-derived ^{13}C in leaf respiration and DSD, drought intensity does determine the dynamics of C translocation as suggested for the leaves.

The increased MRT of recently formed assimilates in the leaves coincides with the delay of the label appearance in soil respiration under drought conditions. This delay is likely linked to the reduced photosynthesis rate and points to a C-source limitation of the drought-stressed beech saplings. This may lead to slower

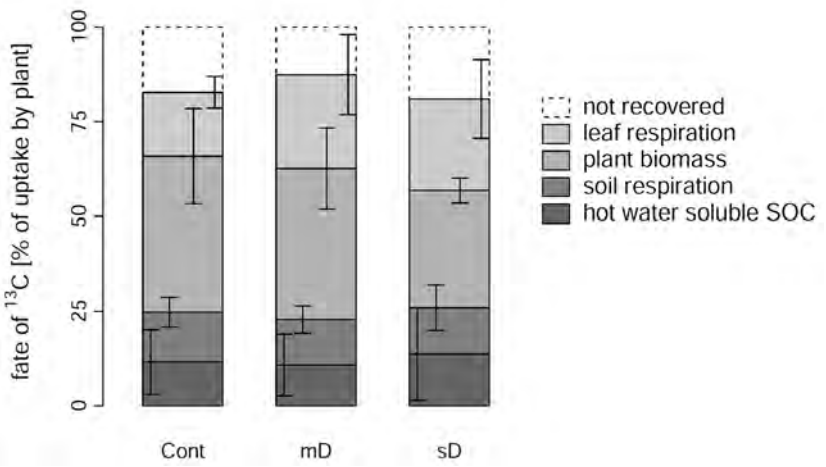

Figure 5.6: Fate of label-derived ^{13}C 12 days after the second pulse label, n = 10, mean±SD. P-values (ANOVA) for treatment differences are given in Tab. 5.2. Treatments: Cont = control, mD = moderate drought, sD = severe drought.

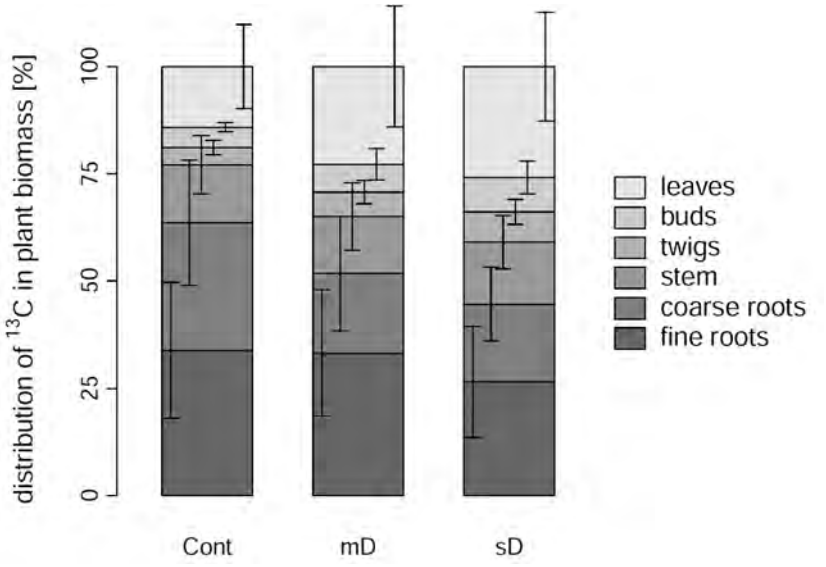

Figure 5.7: Relative distribution of label-derived ^{13}C in different plant compartments 12 days after the second pulse label, n = 10, mean±SD. P-values (ANOVA) for treatment differences are given in Tab. 5.2. Treatments: Cont = control, mD = moderate drought, sD = severe drought.

turnover of carbohydrate pools and, induced by changes in concentration gradients, to smaller carbohydrate transfer rates (Kozlowski, 1992). As soil air was not contaminated by the labeling gas ensured by hermetic sealing of the labeling chambers, it is concluded that the label recovered in soil respiration solely originated from current photosynthesis. Although our sampling intervals do not provide a sufficient temporal resolution to pinpoint the exact time lag, we estimate that the transport of assimilates took at least 15 h longer in beech saplings under treatment sD than in non-stressed saplings. Laser spectroscopy revealed a consistent 11-hour delay of the ^{13}C peak in soil respiration in drought-stressed beech saplings (Barthel et al., 2011). The greater delay in our study may be attributed to larger plants and thus to longer transport paths.

b) Rewetting effects

Photosynthesis

Rewetting resulted in a rapid recovery of net photosynthesis corroborating former studies on this topic (Xu et al. (2010); for beech: Tognetti et al. (1995)). We attribute the recovery from photosynthetic inhibition to stomatal rather than non-stomatal limitation, as the latter might require prolonged recovery periods (Gallé and Feller, 2007), unless leading to irreversible impairment.

MRT of label-derived ^{13}C in leaf respiration and peak in soil respiration

Previously drought-stressed beech saplings did not display persisting drought effects in the MRT of recently formed assimilates in the leaves. Presuming the C demand for aboveground respiration to be higher in such plants, the finding cannot rule out restriction in leaf-to-shoot allocation. However, as there was no delayed ^{13}C peak in soil respiration after rewetting of previously stressed plants, we deduce a fast recovery of the processes involved in assimilate transportation. As the mean residence time of label-derived ^{13}C in leaves of non-stressed beech saplings increased from the first to the second labeling, perhaps incipient autumnal leaf senescence retarding assimilate transport was indicated (Kuptz et al., 2011). It is open as to whether drought-stressed beech saplings behave in a similar way. Nonetheless, the decline in MRT by more than 50 % in sD upon rewetting illustrated an appreciable recovery capacity.

^{13}C-partitioning

As opposed to the respiratory C dynamics and photosynthetic recovery, the ^{13}C mass balance proved after-effects of drought on C partitioning. The increased demand for recently formed assimilates in aboveground respiration of previously drought-stressed plants might be due to repair processes, e.g. repair of embolism or the photosynthetic apparatus (Bréda et al., 2006; McDowell et al., 2008).

In contrast, we did not find indications in soil respiration that drought injury extended to the belowground tree compartments. Neither did drought affect the proportion of ^{13}C recovered by soil extraction. Hence, we conclude that root exudation and root mortality did not differ shortly after the rewetting event. We did not observe differences in fine root biomass within 12 days after rewetting yet we can not exclude enhanced fine root production in later stages of recovery as described by (Olesinski et al., 2011). The recovery rate of ^{13}C of 81 to 88 % did not depend on the treatment from which we conclude that it resulted from diffusive loss of CO_2 during the labeling, via drainage perforation at the bottom of the pots and from stem respiration that was not considered in the mass balance. Based on our measurements, we estimate that ^{13}C emitted by stem respiration did not exceed 2.5 % of total ^{13}C uptake.

Persisting drought effects after rewetting became apparent in the partitioning of ^{13}C in the plant biomass. As there were no significant differences in total plant biomass and root/shoot ratio between the treatments after harvest (Tab. 5.1), the observed pattern of ^{13}C partitioning in different plant organs is considered to be a result of changes in C allocation and is not due to different pool sizes or tree dimensions. Smith and Paul (1988) and Epron et al. (2011) found an increasing amount of recent assimilates to become allocated to the belowground compartments towards the end of the growing season. We assume such behavior to be characteristic for non-stressed plants as they transferred more than 60 % of recently formed assimilates (probably mostly non-structural carbohydrates) to coarse and fine roots. In contrast, previously drought-stressed individuals appear to have invested the C gain in repairing drought injury rather than in filling reserve pools (Bréda et al., 2006; McDowell, 2011). Carbon partitioning and its response to drought stress may not only vary among tree species or provenances but also with tree age. It is well known that the ontogenetic stage of trees, their reached dimensions and the environment of up-growth substantially shape the responsiveness to stress (Kolb and Matyssek, 2001; Hinckley et al., 2011). The micro-climatic environment of seedlings and saplings fundamentally differs from that of mature trees (Johnson et al., 2011). Further, the resource allocation - as a consequence of allometric commonalities - differs for mature and juvenile trees (Thomas, 2011; King et al., 2002; Ishii, 2011). The findings here from juvenile potted trees therefore represent one first step in spatio-temporal up-scaling towards stand-level scenarios of maturing trees (c.f. Kolb and Matyssek, 2001), i.e. principles in responsiveness are presented that await empirical validation beyond the sapling stage and reported growth conditions.

5.6 Conclusions

Drought has been found as a stressor that can intermittently decouple above from belowground C fluxes in plants. Metabolic deceleration may represent a means of extending the resistance to drought by saving assimilates under reduced photosynthesis, and hence, of enhancing the chance of survival. This conclusion appears to be consistent with the fast recovery following rewetting implying that, although drought caused C limitation to the beech saplings, essential C fluxes were maintained. Resistance to drought probably requires processes of repair, which may be one reason of the observed after-effects of drought in C partitioning. We consider the cumulated soil water potential as a proxy for drought stress that correlates with isotopic signatures in leaf and soil respiration, photosynthetic rate and MRT of recently formed assimilates in leaf respiration. Owing to its scalability, DSD might therefore serve as a reference in future drought experiments.

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Appendix

Contributions to the included manuscripts

Record of contributions [%] of each author to the included manuscripts with respect to different aspects with **a** = concept and experimental design, **b** = field and laboratory work, **c** = data evaluation and statistical analysis, **d** = discussion and interpretation of the results and **e** = manuscript preparation.

manuscript	**author**	**a**	**b**	**c**	**d**	**e**
Growth of juvenile beech (*Fagus sylvatica* L.)	Goisser	35	45	55	40	45
upon transplant into a wind-opened	Zang	45	55	45	40	35
spruce stand of heterogeneous	Matzner	5			5	5
light and water conditions	Borken	5			5	5
(Forest Ecology and Management,	Häberle	5			5	5
310:110-119, 2013)	Matyssek	5			5	5
Planted beech saplings (*Fagus sylvatica* L.)	Zang	45	50	75	45	60
under repeated soil drought: high	Goisser	35	50	25	35	20
resilience upon transplantation	Häberle	5			5	
	Matyssek	5			5	5
	Matzner	5			5	5
	Borken	5			5	10
Effects of drought stress on photosynthesis,	Zang	60	80	70	55	50
rhizosphere respiration, and fine root	Goisser	25	20	30	20	25
characteristics of beech saplings:	Häberle				5	5
A rhizotron field study	Matyssek				5	5
(J. of Plant Nutrition and Soil Science,	Matzner	5			5	5
doi: 10.1002/jpln.201300196, 2013)	Borken	10			10	10
Fate of recently fixed carbon in European	Zang	40	45	55	30	45
beech (*Fagus sylvatica* L.) saplings during	Goisser	40	55	45	40	35
drought and subsequent recovery	Häberle				5	
(Tree Physiology,	Grams	10			5	5
doi: 10.1093/treephys/tpt110, 2014))	Matyssek	5			5	5
	Matzner				5	5
	Borken	5			10	5

Further publications of the candidate

Mikutta R., Zang U., Chorover J., Haumaier L. and Kalbitz K. (2011): Stabilization of extracellular polymeric substances (*Bacillus subtilis*) by adsorption to and coprecipitation with Al forms. *Geochimica et Cosmochimica Acta*, 75(11):3135–3154.

von Suchodoletz H., Glaser B., Thrippleton T., Broder T., Zang U., Eigenmann R., Kopp B., Reichert M. and Ludwig Z. (2013): The influence of Saharan dust deposits on La Palma soil properties (Canary Islands, Spain). *Catena*, 103:44–52.

Zang U., Lamersdorf N. and Borken W. (2011): Response of the fine root system in a Norway spruce stand to 13 years of reduced atmospheric nitrogen and acidity input. *Plant and Soil*, 339(1-2):435–445.

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(Eidesstattliche) Versicherungen und Erklärungen

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